JOINT INVENTORS

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APPLICATION FOR UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Nancy K. Mize a citizen of the United States of America, residing at 662 Mountain View Avenue, Mountain View, 94041, in the State of California and Dana A. Haley-Vicente a citizen of the United States of America, residing at 1430 Glenwood Avenue, San Jose, 95125, in the State of California have invented a new and useful NOVEL INTERLEUKIN-1 HY2 MATERIALS AND METHODS, of which the following is a specification.

NOVEL INTERLEUKIN - 1 Hy2
MATERIALS AND METHODS

RELATED APPLICATIONS

This patent application claims priority benefit from Untied States Provisional Application No. 60/245,346 filed November 2, 2001, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to a novel polynucleotide encoding a protein called IL-1 Hy2, which is structurally related to interleukin-1 receptor antagonist protein, along with therapeutic, diagnostic and research utilities for these and related products.

BACKGROUND

Cytokines are involved in inflammation and the immune response, in part through endothelial cell activation. Distinct immune-mediators such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and gamma-interferon (IFN) appear to induce different but partially overlapping patterns of endothelial cell activation including increased procoagulant activity (Bevilaqua (1986) PNAS, 83:4533-4537), PGI and 2 production (Rossi (1985), Science, 229:174-176), HLA antigen expression (Pober (1987) J. Immunol., 138:3319-3324) and lymphocyte adhesion molecules (Carender (1987) J. Immunol., 138:2149-2154). These cytokines are also reported to cause hypotension, vascular hemorrhage, and ischemia (Goldblum et al. 1989, Tracey et al. Science 234:470, 1986). A major toxicity of these and other biological response modifiers is hypotension and vascular leakage (Dvorak (1989) J.N.C.I., 81:497-502).

IL-1 is produced by a number of cell types, including monocyte and macrophages, Langerhans cells, natural killer cells, B cells, T cell leukemic cell lines, neutrophils, endothelial cells, dendritic cells, melanoma cell lines, mesangial cells, astrocytes, glioma cells, microglial cells, fibroblasts and epithelial cells. Two forms of IL-1 have been isolated; IL-1α and IL-1β. They represent the products of two distinct genes and their mature forms are 159 and 153 amino acid proteins, respectively. These molecules are extremely potent and multi-functional cell activators, with a spectrum that encompasses

cells of hematopoietic origin, from immature precursors to differentiated leukocytes, vessel wall elements, and cells of mesenchymal, nervous and epithelial origin. IL-1 also induces production of secondary cytokines, including IL-6, colony stimulating factors (CSFs) and chemokines. IL-1 is active as a hematopoietic growth and differentiation factor; activates endothelial cells in a pro-inflammatory and pro-thrombotic manner (including by inducing production of tissue factor and platelet activating factor); stimulates the release of corticotropin-releasing hormone (CRH) that ultimately causes release of corticosteroids by the adrenals; mediates the acute phase response (including by inducing hepatocyte production of acute phase proteins) and is a central mediator of local and systemic inflammatory reactions that can lead to sepsis and septic shock; is the primary endogenous pyrogen (causing fever); induces slow-wave sleep and anorexia; may play a role in destructive joint and bone diseases (including by inducing production of collagenase by synovial cells and metalloproteinases by chondrocytes); stimulates fibroblast proliferation and collagen synthesis; and may play a role in the pathogenesis of insulin-dependent type I diabetes through its toxicity for insulin-producing beta cells in Langerhans islets.

The IL-1 pathway consists of the two agonists IL-1α and IL-1β, a specific activation system (IL-1 converting enzyme), a receptor antagonist (IL-1Ra) produced in different isoforms and two high affinity receptors. IL-1α and IL-1β bind to two distinct IL-1 receptor types, IL-1 receptor type I (IL-1RI) and IL-1 receptor type II (IL-1RII), both of which are members of the immunoglobulin superfamily of receptors. Both types of receptors are usually coexpressed, although type I is the predominant form in fibroblasts and T cells, while type II is preferentially expressed on B cells, monocytes and neutrophils. IL-1RI and IL-1RII have different affinities for the three ligands of the IL-1 family (IL-1α, IL-1β and IL-1Ra). In particular, IL-1Ra binds to the type I receptor with an affinity similar to that of IL-1α, while IL-1Ra binds to the type II receptor 100-fold less efficiently than the type I receptor. There is evidence indicating that IL-1 induced activities are mediated exclusively via the type I receptor, whereas the type II receptor has no signaling activity and inhibits IL-1 activities by acting as a decoy for IL-1.

IL-1 receptor antagonist (IL-1Ra or IRAP) binds to the IL-1 receptor with affinity similar to that of IL-1 but has no IL-1-like activity, even at very high concentrations, and thus inhibits (antagonizes) the activity of IL-1. The purified IL-1Ra molecule has a

molecular weight of approximately 22 kD and is believed to be glycosylated. It has limited sequence similarity to IL-1α and IL-1β at the amino acid level (19% and 26%, respectively). There appear to be at least two isoforms of IL-1Ra, including a soluble form and an intracellular form generated by an alternative splicing event. IL-1Ra appears to be produced by monocytes, macrophages, neutrophils and fibroblasts; keratinocytes and cells of epithelial origin produce almost exclusively the intracellular form. In humans, the gene for IL-1Ra has been localized to the long arm of chromosome 2, which is the same region where IL-1α and IL-1β, as well as IL-1RI and IL-1RII, are found.

The ability of IL-1 to modify biological responses has been demonstrated in a variety of studies. For example, the administration of IL-1 to rabbits (Wakabayashi et al., FASEB J 1991;5:338; Okusawa et al. J Clin Invest 1988;81:1162; Ohlsson et al., Nature 1990;348:550; Aiura, et al. Cytokine 1991;4:498) and primates (Fischer et al. Am J Physiol 1991;261:R442) has been shown to result in hypotension, tachycardia, lung edema, renal failure, and, eventually, death, depending on the dose. When the serum from the IL-1 treated animals is examined, the elevation of other cytokines is evident, mimicking the levels seen in acute pancreatitis in humans. (Guice et al., J Surg Res 1991;51:495-499; Heath et al., Pancreas 1993;66:41-45) There is a large body of evidence currently available which supports the role of IL-1 as a major mediator of the systemic response to diseases such as sepsis and pancreatitis and as an activator of the remaining members of the cytokine cascade. (Dinarello et al., Arch Surg 1992;127:1350-1353).

IL-1 is a key mediator in the inflammatory response (for reviews, see Dinarello (1991) Blood 77: 1627-1652; Dinarello et al. (1993) New England J. Med. 328:106-113; Dinarello (1994) FASEB J. 8:1314-1325). The importance of IL-1 in inflammation has been demonstrated by the ability of the highly specific IL-1 receptor antagonist protein to relieve inflammatory conditions (for review, see Dinarello (1991) Blood 77: 1627-1652; Dinarello et al. (1993) New England J. Med. 328:106-113; Dinarello (1994) FASEB J. 8:1314-1325; Dinarello (1993) Immunol. Today 14:260-264). Many of the proinflammatory effects of IL-1, such as the upregulation of cell adhesion molecules on vascular endothelia, are exerted at the level of transcriptional regulation. The transcriptional activation by IL-1 of cell adhesion molecules and other genes involved in the inflammatory response appears to be mediated largely by NF- kappa B (Shirakawa et al. (1989) Molc. Cell Biol. 9:2424-2430; Osborn et al., (1989) Proc. Natl. Acad. Sci. USA

86:2336-2340; Krasnow et al., (1991) Cytokine 3:372-379; Collins et al., (1993) Trends Cardiovasc. Med. 3:92-97). In response to IL-1, the NF- kappa B inhibitory factor I kappa B is degraded and NF- kappa B is released from its inactive cytoplasmic state to localize within the nucleus where it binds DNA and activates transcription (Liou et al. (1993) Curr. Opin. Cell Biol. 5:477-487; Beg et al., (1993) Mol. Cell. Bid. 13:3301-3310).

IL-1 is also a mediator of septic shock . Septic shock, a life-threatening complication of bacterial infections, affects 150,000 to 300,000 patients annually in the United States (Parrillo, J. E. (1989), Septic Shock in Humans: Clinical Evaluation, Pathogenesis, and Therapeutic Approach (2nd ed.) In: Textbook of Critical Care Shoemaker, et al., editors, Saunders Publishing Co., Philadelphia, Pa., pp. 1006). The cardiovascular collapse and multiple metabolic derangements associated with septic shock are due largely to bacterial endotoxin (ET), which has been shown to elicit a septic shock-like condition when administered to animals (Natanson, et al. (1989), Endotoxin and Tumor Necrosis Factor Challenges in Dogs Simulate the Cardiovascular Profile of Human Septic Shock, J. Exp. Med. 169:823). Thus, there is a great need for modulators of IL-1 which may be useful for modulating inflammation and the immune response.

SUMMARY OF THE INVENTION

The compositions of the present invention include novel isolated polypeptides, in particular, novel human Interleukin-1 Hy2 (IL-1 Hy2) proteins and active variants thereof, isolated polynucleotides encoding such polypeptides, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, antisense polynucleotide molecules, and antibodies that specifically recognize one or more epitopes present on such polypeptides, as well as hybridomas producing such antibodies.

The compositions of the present invention additionally include vectors, including expression vectors, containing the polynucleotides of the invention, cells genetically engineered to contain such polynucleotides and cells genetically engineered to express such polynucleotides.

The polynucleotides of the invention include naturally occurring or wholly or partially synthetic DNA, e.g., cDNA and genomic DNA, and RNA, e.g., mRNA. The

isolated polynucleotides of the invention include, but are not limited to, a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NOS: 2, 4 or 13. The isolated polynucleotides of the invention further include, but are not limited to, a polynucleotide comprising the nucleotide sequence of SEQ ID NOS: 1, 12 or 14; a polynucleotide comprising the full length protein coding sequence of SEQ ID NOS: 1, 12 or 14; and a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of SEQ ID NOS: 1, 12 or 14. The polynucleotides of the present invention also include, but are not limited to, polynucleotides that encode polypeptides with IL-1 Hy2 activity and that hybridize under stringent hybridization conditions to the complement of (a) the nucleotide sequence of SEQ ID NOS: 1, 12 or 14, or (b) a nucleotide sequence encoding the amino acid sequence of SEQ ID 2, 4 or 13; a polynucleotide which is an allelic variant of any polynucleotide recited above; a polynucleotide which encodes a species homologue of any of the proteins recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptide having an amino acid sequence of SEQ ID NOS: 2, 4 or 13.

The polynucleotides of the present invention still further include, but are not limited to, a polynucleotide comprising the nucleotide sequence of the cDNA insert of clone pIL-1Hy2 deposited on May 21, 1999 under Accession No. PTA-96 with the American Type Culture Collection (ATCC; 10801 University Blvd., Manassas, Virginia, 20110-2209, U.S.A.) or an IL-1 Hy2 protein coding portion thereof, such as the full length protein coding sequence or the mature protein coding sequence.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above.

A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or identifying information of each sequence can be provided on a nucleic acid array. In one embodiment, segments of sequence information are provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array can be designed to detect nucleic acids that are perfectly complementary (full-match) or mismatched to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising the amino acid sequence of SEQ ID NOS: 2, 4 or 13, or the

amino acid sequence encoded by the cDNA insert of clone pIL-1Hy2, or a portion thereof corresponding to the full length or mature protein. Polypeptides of the invention also include polypeptides with IL-1 Hy2 activity that are encoded by (a) polynucleotides encoding SEQ ID NOS: 2 or 13 (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or immunologically active variants of the IL-1Ra protein sequence of SEQ ID NOS: 2, 4 or 13 and "substantial equivalents" thereof (e.g., with 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% amino acid identity) that retain IL-1 Hy2 activity, preferably IL-1 antagonist activity, are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (e.g. host cells) of the invention.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

The invention also relates to methods for producing polypeptides of the invention comprising growing a culture of the cells of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the protein from the cells or the culture medium. Preferred embodiments include those in which the protein produced by such process is a mature form of the protein.

Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers for PCR, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of anti-sense DNA or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, e.g., in situ hybridization.

In other exemplary embodiments, the polynucleotides are used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide. The polypeptides of the invention can also be used as molecular weight markers, and as a food supplement. Transgenic animals with altered expression of the polypeptides of the invention (i.e. knock out animals or animals overexpressing IL-1 Hy2) are also contemplated.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

In particular, the polypeptides and polynucleotides of the invention can be utilized, for example, as part of methods for the prevention and/or treatment of IL-1, IL-18 and/or IL-12 mediated disorders including disorders involving sepsis (and associated conditions such as fever, tachycardia, tachypnea, cytokine overstimulation, increased vascular permeability, hypotension, complement activation, disseminated intravascular coagulation, anemia, thrombocytopenia, leukopenia, pulmonary edema, adult respiratory distress syndrome, intestinal ischemia, renal insufficiency and failure, metabolic acidosis and multiorgan dysfunction syndrome), endotoxic shock, cytokine induced shock, thrombosis, acute pancreatitis, rheumatoid or reactive arthritis, chronic inflammatory arthitis, vasculitis, lupus, immune complex glomerulonephritis, pancreatic cell damage from diabetes mellitus type 1, allograft and xenograft transplantation, graft versus host disease, inflammatory bowel disease, inflamation associated with pulmonary disease, other autoimmune disease or inflammatory disease, an antiproliferative agent such as for acute or chronic myelogenous leukemia, ovarian carcinoma, or in the prevention of premature labor secondary to intrauterine infections, bone degenerative diseases such as osteoporosis, and neurodegenerative disorders such as Alzheimer disease.

Concurrent administration of other agents that inhibit the production or activity of IL-1 (such as GM-CSF, IL-4, IL-10, IL-13 and transforming growth factor-beta) or other anti-inflammatory agents (such as IL-1Ra, IL-1Ra-like IL-1Hy1 proteins described in co-owned, co-pending U.S. application serial no. 09/287,210 filed April 5, 1999,

incorporated herein by reference, anti-TNF, corticosteroids, immunosuppressive agents) is also contemplated according to the invention.

The methods of the present invention further relate to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample. Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders as recited above and for the identification of subjects exhibiting a predisposition to such conditions. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds that modulate (i.e., increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited above. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (e.g., bind to) the polypeptides of the invention.

The methods of the invention also include methods for the treatment of disorders as recited above which may involve the administration of such compounds to individuals exhibiting symptoms or tendencies related to disorders as recited above. In addition, the invention encompasses methods for treating diseases or disorders as recited above by administering compounds and other substances that modulate the overall activity of the target gene products. Compounds and other substances can effect such modulation either on the level of target gene expression or target protein activity.

The invention further provides a method of treating an inflammatory disease state mediated by IL-18 comprising administering to a subject in need thereof an amount of an IL-1 Hy2 polynucleotide, polypeptide or agonist effective to inhibit IL-18 activity. Also provided are in vitro and in vivo methods of inhibiting IL-18 activity.

Three-dimensional modeling data has suggested that the predicted three-dimensional structure of IL-1 Hy2 closely resembles the three-dimensional structure of IL-1β. This data indicates that IL-1 Hy2 may function as a low affinity agonist to the IL-1 receptor in the absence of accessory protein. Therefore, IL-1 Hy2 may induce pro-inflammatory physiological effects similar to IL-1 β and plays a role in enhancing inflammation related pathological conditions. On the other hand, experimental results

indicate that IL-1 Hy2 is an antagonist of the IL-1 receptor and this is supported by the presence of Lys145 which is an important residue for biological activity.

The invention provides for antagonists, agonists and modulators of IL-1 Hy2, such as antibodies, antisense oligonucleotides, small molecules, peptides and derivatives thereof which reduce IL-1 Hy2 binding interactions with or activation of the IL-1 receptor. The invention also provides for methods of screening for antagonists and modulators of IL-1 Hy2 and methods of treating pathological conditions associated with inflammation by administering IL-1 Hy2 antagonists or modulators thereof.

The predicted three-dimensional structure of IL-1 Hy2 recited herein provides a basis for rationally designing IL-1 Hy2 modulators (such as antagonists and agonists) which specifically associate with the amino acids predicted to interact with a receptor such as IL-1 receptor. Such residues include Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105, Tyr147 and other amino acids of SEQ ID NO: 2 within about 2-12 Å, preferably within 7 Å, and more preferably within 5 Å, that may interact with these amino acids and/or contribute to the three-dimensional conformation of the receptor binding residues. In addition, the predicted three-dimensional structure will allow for the creation of IL-1 Hy2 polypeptide mutants that have similar, increased, decreased or different biological activity compared to wild type IL-1Hy2.

Therefore, the invention provides for a polypeptide comprising an amino acid sequence comprising two or more receptor binding residues substantially defined by structural coordinates of amino acids Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55, Gly92, Gly93, Gln103, Ser105 and Tyr147 of SEQ ID NO: 2 as set forth in Tables II or III, and said polypeptide capable of binding IL-1 receptor. These polypeptides include those which have a root mean squared deviation from the structural coordinates set forth in Tables II or III within 2-12 Å, preferably within 7 Å, or more preferably within 5 Å, those which have an basic residue at the position corresponding to 145 of SEQ ID NO: 2, such as arginine, lysine, and histidine. These polypeptides also include those taht are less than 95% identical, more preferably less than 85% identical over the entire length of S ID NO: 2. The invention also provides for polypeptides that comprises two or more receptor binding residues substantially defined by structural coordinates of amino acids Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105

and Tyr147 of SEQ ID NO: 2 as set forth in Tables II or III, and said polypeptide capable of binding IL-1 receptor but the portion outside of the IL-1 receptor binding region has a three-dimensional conformation substantially different from that of IL-1 Hy2 of SEQ ID NO: 2. The invention also provides for a method of treating a pathological condition, such as psoriasis, characterized by aberrant expression or activity of IL-1 receptor comprising administering to a patient a therapeutically effective amount any one of these polypeptides.

The invention further provides for an IL-1 Hy2 polypeptide variant comprising at least one modification wherein an amino acid residue selected from the group consisting of Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105, Lys145 and Tyr147 is replaced with a different amino acid, and wherein said IL-1 Hy2 polypeptide variant exhibits increased or deceased binding to IL-1 receptor compared to IL-1 Hy2 of SEQ ID NO: 2. The modification contemplates replacing at least one amino acid with a conservative substitution.

Another embodiment of this invention provides a machine-readable storage medium comprising a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using said data, is capable of displaying a graphical three-dimensional structure corresponding to IL-Hy1 (including IL-1 Hy2 variants), particularly as defined herein with reference to receptor binding residues, accessory protein binding residues and other residues important to IL-1 Hy2 biological function. For example, the machine readable storage medium includes a three-dimensional representation which is substantially defined by the structural coordinates of amino acids Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105, Lys145 and Tyr147 of SEQ ID NO: 2 as set forth in Tables II or III.

The invention provides for a computer comprising memory containing the three-dimensional representation of IL-1 Hy2 or a portion of IL-1 Hy2 that includes the IL-1 receptor binding regions of IL-1 Hy2. These computers include those comprising memory of a three-dimensional representation that is substantially defined by structural coordinates of IL-1 Hy2 amino acids Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105 and Tyr147 of SEQ ID NO: 2 as set forth in Tables II or III or those wherein the IL-1 receptor binding region has a root mean square deviation from the structural coordinates set forth in Tables II or III of amino acids Met 6,

Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105 and Tyr147 within 2-12 Å, preferably within 7 Å, or more preferably within 5 Å. The invention also provides for a computer comprising a computer readable storage medium, which is a data storage material coded with machine readable data, wherein said data includes the three-dimensional representation of IL-1 Hy2 or a portion of IL-1 Hy2 that includes the IL-1 receptor binding regions of IL-1 Hy2 described herein.

Another embodiment of the invention provides for methods of identifying potential modulators of IL-1 Hy2 biological activity using a three-dimensional structure of IL-1 Hy2 substantially defined by the structural coordinates of two or more IL-1 Hy2 (SEQ ID NO: 2) amino acids Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105 and Tyr147 as set forth in Tables II or III to design or select potential modulators and contacting said modulators with IL-1 Hy2 in the presence of IL-1 receptor to test the ability of said potential modulator to modulate the interaction between IL-1 Hy2 and IL-1 receptor. These methods include selecting modulators using a computer for interaction with the three-dimensional structure of IL-1 Hy2. These methods also include contacting said potential modulator with an IL-1 Hy2 mutant, which exhibits reduced binding to IL-1 receptor compared to wild type IL-1 Hy2 (SEQ ID NO: 2), to test the ability of the modulator to modulate the interaction between the IL-1 Hy2 mutant and IL-1 receptor. These methods will includes the use of mutants which comprise at least one modification wherein an amino acid residue selected from the group consisting of Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105 and Tyr147 is replaced with a different amino acid, and wherein said IL-1 Hy2 polypeptide variant exhibits decreased binding to IL-1 receptor compared to IL-1 Hy2 of SEQ ID NO: 2.

The invention provides for methods of treating pathological condition characterized by aberrant expression or activity of IL-1 Hy2, comprising administering to a patient a therapeutically effective amount of a non-peptidyl compound that is a biological modulator of IL-1 Hy2 interaction with IL-1 receptor, said compound containing one or more moieties that mimic one or more of the IL-1 Hy2 amino acids of SEQ ID NO: 2 selected from the group consisting of Met 6, Arg 8, Lys13, Gln17, Asp31, Val33, Pro46, Val55,Gly92, Gly93, Gln103, Ser105, Lys145 and Tyr147 and as set forth in Tables II or III.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B show an alignment of the amino acid sequence of IL-1 Hy2 (SEQ ID NO. 2) with the sequences of IL-1Hy1 (described in co-owned, co-pending U.S. Serial No. 09/287,210 filed April 5, 1999), rat IL-1Ra, pig IL-1Ra, secreted human IL-1Ra (Hu sIL-1Ra) and intracellular human IL-1Ra (Hu icIL-1Ra), SEQ ID NOS: 5-9, respectively. In these figures, A- Alanine; R- Arginine; N- Asparagine; D- Aspartic Acid; C- Cysteine; E- Glutamic Acid; Q- Glutamine; G- Glycine; H- Histidine; I- Isoleucine; L- Leucine; K- Lysine; M- Methionine; F- Phenylalanine; P- Proline; S- Serine; T- Threonine; W- Tryptophan; Y- Tyrosine; V- Valine; X - any of the twenty amino acids. Gaps are presented as dashes. Amino acid numbers for all sequences are labelled accordingly. Boxed residues indicate consensus or conserved sequence.

FIG. 2 sets forth SEQ ID NO: 12 which represents the predicted cDNA sequence based on the human genomic sequence of IL-1 Hy2 .

FIG. 3 sets forth SEQ ID NO: 13 which represents the human amino acid sequence encoded by the longer open reading frame of SEQ ID NO: 12 which is an alternative form of the IL-1 Hy2 polypeptide.

FIG. 4 sets forth SEQ ID NO: 14 which represents the cDNA sequence of human IL-1 Hy2 clones which extends the 5' region of SEQ ID NO: 1.

FIG.5 shows ribbon diagrams of the IL-1 Hy2 predicted three-dimensional structural model superimposed with the IL-1 Ra (top panel) average NMR structure and IL-1 β (low panel) crystal structure. This figure demonstrates that IL-1 Hy2 is more structurally similar to IL-1β than IL-1 Ra.

FIG.6 shows the alignment based on secondary structure of the amino acid sequences of IL-1 Ra and IL-1 Hy2 and indicates the residues involved in receptor interaction and critical function. An amino acid symbol between the two sequences indicates identity and "+" indicates similarity. The amino acids in bold are within the receptor binding region. A critical function amino acid is underlined. The arrows indicate the location of the β-strands within the three-dimensional structure.

FIG. 7 shows the alignment based on secondary structure of the amino acid sequences of IL-1 β and IL-1 Hy2 and indicates the residues involved in receptor interaction and function. An amino acid symbol between the two sequences indicates identity and "+" indicates similarity. The amino acids in bold are within the receptor

binding region. A critical function amino acid is underlined. The arrows indicate the location of the β-strands within the three-dimensional structure.

DETAILED DESCRIPTION OF THE INVENTION

1. DEFINITIONS

The term "nucleotide sequence" refers to a heteropolymer of nucleotides or the sequence of these nucleotides. The terms "nucleic acid" and "polynucleotide" are also used interchangeably herein to refer to a heteropolymer of nucleotides. Generally, nucleic acid segments provided by this invention may be assembled from fragments of the genome and short oligonucleotide linkers, or from a series of oligonucleotides, or from individual nucleotides, to provide a synthetic nucleic acid which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon, or a eukaryotic gene.

The terms "oligonucleotide fragment" or a "polynucleotide fragment", "portion," or "segment" is a stretch of polypeptide nucleotide residues which is long enough to use in polymerase chain reaction (PCR) or various hybridization procedures to identify or amplify identical or related parts of mRNA or DNA molecules.

The terms "oligonucleotides" or "nucleic acid probes" are prepared based on the polynucleotide sequences provided in the present invention. Oligonucleotides comprise portions of such a polynucleotide sequence having at least about 15 nucleotides and usually at least about 20 nucleotides. Nucleic acid probes comprise portions of such a polynucleotide sequence having fewer nucleotides than about 6 kb, usually fewer than about 1 kb. After appropriate testing to eliminate false positives, these probes may, for example, be used to determine whether specific mRNA molecules are present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250).

The term "probes" includes naturally occurring or recombinant or chemically synthesized single- or double-stranded nucleic acids. They may be labeled by nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory,

NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent conditions (i.e., hybridization to filter-bound DNA under in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C), and moderately stringent conditions (i.e., washing in 0.2xSSC/0.1% SDS at 42°C).

In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary stringent hybridization conditions include washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos).

The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, e.g., *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

The term "recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed. This term also means host cells which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Recombinant expression systems as defined herein will express polypeptides or proteins endogenous to the cell upon induction of the regulatory elements linked to the endogenous DNA segment or gene to be expressed. The cells can be prokaryotic or eukaryotic.

The term "open reading frame," ORF, means a series of nucleotide triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

The term "expression modulating fragment," EMF, means a series of nucleotides which modulates the expression of an operably linked ORF or another EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs are fragments which induce the expression or an operably linked ORF in response to a specific regulatory factor or physiological event.

As used herein, an "uptake modulating fragment," UMF, means a series of nucleotides which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described below.

The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence.

The term "active" refers to those forms of the polypeptide which retain the biologic and/or immunologic activities of any naturally occurring polypeptide. According to the invention, the term "biologically active" with reference to IL-1 Hy2 means that the polypeptide retains at least one of the biological activities, preferably the IL-1 antagonist activity, of human IL-1 Hy2, while the term "immunologically active" with reference to IL-1 Hy2 means that the polypeptide retains at least one of the immunologic or antigenic activities of human IL-1 Hy2.

The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (e.g., with radionuclides or various enzymes), pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

The term "variant" (or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, such as IL-1 antagonistic activity, may be found by comparing the sequence of the particular polypeptide with that of homologous human or other mammalian peptides e.g. IL-1Ra, IL-1Hy1, or IL-1, and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, i.e., conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline,

phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Alternatively, where alteration of function is desired, insertions, deletions or non-conservative alterations can be engineered to produce altered polypeptides. Such alterations can, for example, alter one or more of the biological functions or biochemical characteristics of the polypeptides of the invention. For example, such alterations may change polypeptide characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Further, such alterations can be selected so as to generate polypeptides that are better suited for expression, scale up and the like in the host cells chosen for expression. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

As used herein, "substantially equivalent" can refer both to nucleotide and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and subject sequences. Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about 20% (i.e., the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.2 or less). Such a sequence is said to have 80% sequence identity to the listed sequence. In one embodiment, a substantially equivalent, e.g., mutant, sequence of the invention varies from a listed sequence by no more than 10% (90% sequence identity); in a variation of this embodiment, by no more than 5% (95% sequence identity); and in a further variation of this embodiment, by no more than 2% (98% sequence identity). Substantially equivalent, e.g., mutant, amino acid sequences according to the invention generally have at least 95% sequence identity with a

listed amino acid sequence, whereas substantially equivalent nucleotide sequence of the invention can have lower percent sequence identities, taking into account, for example, the redundancy or degeneracy of the genetic code. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. For the purposes of determining equivalence, truncation of the mature sequence (e.g., via a mutation which creates a spurious stop codon) should be disregarded. Sequence identity may be determined, e.g., using the Jotun Hein method.

Nucleic acid sequences encoding such substantially equivalent sequences, e.g., sequences of the recited percent identities, can routinely be isolated and identified via standard hybridization procedures well known to those of skill in the art.

Where desired, an expression vector may be designed to contain a "signal or leader sequence" which will direct the polypeptide through the membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, often at least about 7 amino acids, typically at least about 9 to 13 amino acids, and, in various embodiments, at least about 17 or more amino acids. To be active, any polypeptide must have sufficient length to display biologic and/or immunologic activity.

Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

The term "activated" cells as used in this application are those which are engaged in extracellular or intracellular membrane trafficking, including the export of neurosecretory or enzymatic molecules as part of a normal or disease process.

The term "purified" as used herein denotes that the indicated nucleic acid or polypeptide is present in the substantial absence of other biological macromolecules, e.g., polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at least 95% by weight, more preferably at least 99.8% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

The term "isolated" as used herein refers to a nucleic acid or polypeptide separated from at least one other component (e.g., nucleic acid or polypeptide) present with the nucleic acid or polypeptide in its natural source. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other component normally present in a solution of the same. The terms "isolated" and "purified" do not encompass nucleic acids or polypeptides present in their natural source.

The term "infection" refers to the introduction of nucleic acids into a suitable host cell by use of a virus or viral vector.

The term "transformation" means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extra chromosomal element, or by chromosomal integration.

The term "transfection" refers to the taking up of an expression vector by a suitable host cell, whether or not any coding sequences are in fact expressed.

The term "intermediate fragment" means a nucleic acid between 5 and 1000 bases in length, and preferably between 10 and 40 bp in length.

The term "secreted" includes a protein that is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence when it is expressed in a suitable host cell. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g., receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins which are transported across the membrane of the endoplasmic reticulum. "Secreted" proteins are also intended to include proteins containing non-typical signal sequences (e.g. Interleukin-1 Beta, see Krasney, P.A. and Young, P.R. (1992) Cytokine 4(2): 134 -143) and factors released from damaged cells (e.g. Interleukin-1 Receptor Antagonist, see Arend, W.P. et. al. (1998) Annu. Rev. Immunol. 16:27-55)

Each of the above terms is meant to encompasses all that is described for each, unless the context dictates otherwise.

NUCLEIC ACIDS AND POLYPEPTIDES OF THE INVENTION

Nucleotide and amino acid sequences of the invention are reported below. Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein-IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms (for example, without a signal sequence or precusor sequence) of the disclosed proteins. The full-length form of the such proteins is identified in the sequence listing by translation of the nucleotide sequence of each disclosed clone. The mature form of such protein may be obtained by expression of the disclosed full-length polynucleotide in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein is also determinable from the amino acid sequence of the full-length form. Where protein of the present invention is membrane bound, soluble forms of the protein are also provided. In such forms part or all of the regions causing the protein to be membrane bound are deleted so that the protein is fully secreted from the cell in which it is expressed.

The present invention also provides genes corresponding to the cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other

sources of genomic materials. Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species. The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides. The compositions of the present invention include isolated polynucleotides, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, novel isolated polypeptides, and antibodies that specifically recognize one or more epitopes present on such polypeptides. Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species. The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 sets forth one preferred nucleotide sequence of IL-1 Hy2 which contains a protein coding region from nucleotides 54 through 509.

SEQ ID NO: 2 sets forth an amino acid sequence encoded by SEQ ID NO: 1.

SEQ ID NO: 3 sets forth a nucleotide sequence identical to SEQ ID NO: 1 except the protein coding region spans nucleotides 3 through 509.

SEQ ID NO: 4 sets forth the amino acid sequence encoded by SEQ ID NO: 3.

SEQ ID NOS: 5-9 set forth the amino acid sequences of human IL-1 Hy1. rat IL-1Ra, pig IL-1Ra, human IL-1Ra and intracellular human IL-1Ra, respectively.

SEQ ID NO: 14 sets forth an extended cDNA sequence of a human IL-1 Hy2 clone which is longer than SEQ ID NO: 1 but encodes the same 152 amino acid polypeptide (SEQ ID NO: 2).

SEQ ID NO: 15 sets forth the genomic DNA sequence of human IL-1 Hy2.

SEQ ID NO: 12 sets forth the predicted cDNA sequence based on the human genomic sequence of IL-Hy2.(SEQ ID NO: 15) and differs from SEQ ID NO: 14 at position 279 (C→T).

SEQ ID NO: 13 sets forth the 200 amino acid sequence encoded by the longer open reading frame of SEQ ID NO: 12.

SEQ ID NO: 16 sets forth the genomic DNA sequence of murine IL-1 Hy2.

SEQ ID NO: 17 sets forth the predicted murine cDNA sequence based on the mouse genomic sequence of SEQ ID NO: 16.

SEQ ID NO: 18 sets forth the deduced amino acid sequence of murine IL-1 Hy2 polypeptide.

2. NUCLEIC ACIDS OF THE INVENTION

The isolated polynucleotides of the invention include, but are not limited to, a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NOS: 2, 4 or 13. A preferred nucleic acid sequence is set forth in SEQ ID NO: 1 (which is identical to SEQ ID NO: 3 except for the identification of the protein coding region, which is nucleotides 54 through 509 for SEQ ID NO: 1 and nucleotides 3 through 509 for SEQ ID NO: 3).

There are two alternative open reading frames in SEQ ID NO: 1. Resequencing of the 5′ region of the IL-1 Hy2 cDNA resulted in SEQ ID NO: 14 which includes the shorter open reading frame of SEQ ID NO: 1 and extends its 5′ sequence. The predicted amino acid sequence based upon the shorter open reading frame of SEQ ID NO: 14 is shown in SEQ ID NO: 2. The predicted cDNA sequence based on the genomic DNA sequence is set forth as SEQ ID NO: 12, which contains a C→T change that results in an alternative upstream initiating methionine which extends the open reading frame of SEQ ID NO: 3. The predicted amino acid sequence based on the longer open reading frame is shown in SEQ ID NO: 13.

The isolated polynucleotides of the invention further include, but are not limited to a polynucleotide comprising the nucleotide sequence of SEQ ID NOS: 1, 12 or 14; a polynucleotide comprising the full length protein coding sequence of SEQ ID NOS: 1, 12 or 14; and a polynucleotide comprising the nucleotide sequence of the mature protein

coding sequence of SEQ ID NOS: 1, 12 or 14. The polynucleotides of the present invention also include, but are not limited to, polynucleotides that encode polypeptides with IL-1 Hy2 activity and that hybridize under stringent hybridization conditions to the complement of either (a) the nucleotide sequence of SEQ ID NOS: 1, 12 or 14, or (b) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2, 4 or 13; a polynucleotide which is an allelic variant of any polynucleotide recited above; a polynucleotide which encodes a species homologue of any of the proteins recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptide of SEQ ID NOS: 2, 4 or 13.

The polynucleotides of the present invention still further include, but are not limited to, a polynucleotide comprising the nucleotide sequence of the cDNA insert of clone pIL-1Hy2 or an IL-1 Hy2 protein coding portion thereof, such as the full length protein coding sequence or the mature protein coding sequence.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above.

The polynucleotides of the invention also provide polynucleotides including nucleotide sequences that are substantially equivalent to the polynucleotides recited above. Polynucleotides according to the invention can have at least about 65%, more typically at least about 70%, 75%, 80%, 85% or 90%, and even more typically at least about 95%, sequence identity to a polynucleotide recited above. The invention also provides the complement of the polynucleotides including a nucleotide sequence that has at least about 80%, more typically at least about 90%, and even more typically at least about 95%, sequence identity to a polynucleotide encoding a polypeptide recited above. The polynucleotide can be DNA (genomic, cDNA, amplified, or synthetic) or RNA. Methods and algorithms for obtaining such polynucleotides are well known to those of skill in the art and can include, for example, methods for determining hybridization conditions which can routinely isolate polynucleotides of the desired sequence identities.

A polynucleotide according to the invention can be joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polypeptides include an assortment of vectors, e.g., plasmids, cosmids, lambda phage derivatives, phagemids, and

the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The sequences falling within the scope of the present invention are not limited to the specific sequences herein described, but also include allelic variations thereof. Allelic variations can be routinely determined by comparing the sequence provided in SEQ ID NOS: 1, 12 or 14, or a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NOS: 1, 12 or 14 with a sequence from another isolate of the same species. Example 2 shows that several allelic variants exist, some of which result in changes in the encoded polypeptide sequence.

To accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another which encodes the same amino acid is expressly contemplated. Any specific sequence disclosed herein can be readily screened for errors by resequencing a particular fragment, such as an ORF, in both directions (i.e., sequence both strands).

The present invention further provides recombinant constructs comprising a nucleic acid having the sequence of SEQ ID NOS: 1, 12 or 14; or a fragment thereof or any other polynucleotides of the invention. In one embodiment, the recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a nucleic acid having the sequence of SEQ ID NOS: 1, 12 or 14; or a fragment thereof is inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. For vectors comprising the EMFs and UMFs of the present invention, the vector may further comprise a marker sequence or heterologous ORF operably linked to the EMF or UMF. Large numbers of suitable vectors and promoters are known to those of skill in the art and

are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a

fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced or derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequences that hybridize under stringent conditions to a fragment of the DNA sequence of SEQ ID NOS: 1, 12 or 14, which fragment is greater than about 10 bp, preferably 20-50 bp, and even greater than 100 bp. In accordance with the invention, polynucleotide sequences which encode the novel nucleic acids, or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of that nucleic acid, or a functional equivalent thereof, in appropriate host cells.

The nucleic acid sequences of the invention are further directed to sequences which encode variants of the described nucleic acids. These amino acid sequence variants

may be prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. The amino acid sequence variants of the nucleic acids are preferably constructed by mutating the polynucleotide to give an amino acid sequence that does not occur in nature. These amino acid alterations can be made at sites that differ in the nucleic acids from different species (variable positions) or in highly conserved regions (constant regions). Sites at such locations will typically be modified in series, e.g., by substituting first with conservative choices (e.g., hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant choices (e.g., hydrophobic amino acid to a charged amino acid), and then deletions or insertions may be made at the target site. Amino acid sequence deletions generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 amino residues, preferably from 1 to 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells.

In a preferred method, polynucleotides encoding the novel nucleic acids are changed via site-directed mutagenesis. This method uses oligonucleotide sequences that encode the polynucleotide sequence of the desired amino acid variant, as well as a sufficient adjacent nucleotide on both sides of the changed amino acid to form a stable duplex on either side of the site of being changed. In general, the techniques of site-directed mutagenesis are well known to those of skill in the art and this technique is exemplified by publications such as, Edelman et al., DNA 2:183 (1983). A versatile and efficient method for producing site-specific changes in a polynucleotide sequence was published by Zoller and Smith, Nucleic Acids Res. 10:6487-6500 (1982). PCR may also be used to create amino acid sequence variants of the novel nucleic acids. When small amounts of template DNA are used as starting material, primer(s) that differs slightly in sequence from the corresponding region in the template DNA can generate the desired amino acid variant. PCR amplification results in a population of product DNA fragments

that differ from the polynucleotide template encoding the polypeptide at the position specified by the primer. The product DNA fragments replace the corresponding region in the plasmid and this gives the desired amino acid variant.

A further technique for generating amino acid variants is the cassette mutagenesis technique described in Wells et al., Gene 34:315 (1985); and other mutagenesis techniques well known in the art, such as, for example, the techniques in Sambrook et al., supra, and Current Protocols in Molecular Biology, Ausubel et al. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used in the practice of the invention for the cloning and expression of these novel nucleic acids. Such DNA sequences include those which are capable of hybridizing to the appropriate novel nucleic acid sequence under stringent conditions.

Furthermore, knowledge of the DNA sequence provided by the present invention allows for the modification of cells to permit, or increase, expression of endogenous IL-1 Hy2 polypeptides. Cells can be modified (e.g., by homologous recombination) to provide increased IL-1 Hy2 expression by replacing, in whole or in part, the naturally occurring IL-1 Hy2 promoter with all or part of a heterologous promoter so that the cells express IL-1 Hy2 polypeptides at a higher level. The heterologous promoter is inserted in such a manner that it is operatively linked to IL-1 Hy2 encoding sequences. See, for example, PCT International Publication No. WO94/12650, PCT International Publication No. WO92/20808, and PCT International Publication No. WO91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., ada, dhfr, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the IL-1 Hy2 coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the IL-1 Hy2 coding sequences in the cells.

The polynucleotides of the present invention also make possible the development, through, e.g., homologous recombination or knock out strategies, of animals that fail to express functional IL-1 Hy2 polypeptides or that express a variant of a IL-1 Hy2

polypeptide. Such animals are useful as models for studying the *in vivo* activities of IL-1 Hy2 polypeptides as well as for studying modulators of IL-1 Hy2 polypeptides.

Polynucleotides of the invention can also be used to induce immune responses. For example, as described in Fan et al., Nat. Biotech. 17:870-872 (1999), incorporated herein by reference, nucleic acid sequences encoding a polypeptide may be used to generate antibodies against the encoded polypeptide following topical administration of naked plasmid DNA or following injection, and preferably intramuscular injection of the DNA. The nucleic acid sequences are preferably inserted in a recombinant expression vector and may be in the form of naked DNA.

3. HOSTS

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. *et al., Basic Methods in Molecular Biology* (1986)). The host cells containing one of polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or

other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell tines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include

Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting, including polyadenylation signals. mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, e.g., inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable

marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

4. POLYPEPTIDES OF THE INVENTION

SEQ ID NO. 1 encodes the IL-1 Hy2 polypeptide sequence of SEQ ID NOS: 2, 4 and 13. An amino acid alignment of SEQ ID NO. 2 with human secreted IL-1Ra, human intracellular IL-1Ra and human IL-1Hy1, as well as rat and pig IL-1Ra, is shown in FIG. 1. SEQ ID NO. 2 displays significant amino acid homology with human IL-1Ra and IL-1 Hy1 (41.4% and 45% sequence identity, respectively, using the Jotan Hein method), and thus represents a novel molecule within the IL-1Ra family. The sequence similarities among the three proteins and the localization of the IL-1 Hy2 gene to chromosome 2, where other proteins of the IL-1 system are located, indicate that IL-1 Hy2 is involved in the IL-1 system and may play some common biological roles as IL-1Ra and IL-1Hy1, e.g., acting as an IL-1 antagonist. Additional IL-1Hy2 family members can be identified using SEQ ID NOS: 1, 12 or 14 as a molecular probe.

Interleukin-1 has pleiotropic biological activities many of which adversely affect the organism, it would be expected that the molecule must be tightly regulated if it is not to be injurious. Indeed, there are several reports of Interleukin-1 inhibitors that regulate

the action of Interleukin-1. Interleukin-1 inhibitory activity has been reported in monocyte conditioned medium, wherein the monocytes are grown on adherent immune complexes. Arena, W. P., et al., 1985, Journal of Immun., 134:3868. Additionally, an inhibitor has been reported to be present urine. Seckinger, P., et al., 1987, Journal of Immun., 139:1546. Lastly, a protein inhibitor, purified and cloned, that has interleukin-1 receptor antagonist activity has been reported. Hannum, et at., 1990, Nature, 343:336, and Eisenberg, S., et al., 1990, Nature, 343:341.

It is thought that the Interleukin-1 inhibitor present in urine, and which has been partially purified and characterized by Seckinger, P. et al., and Seckinger, P., et al., 1987, Journal of Immun., 139:1541 is similar, if not identical to the cloned Interleukin-1 receptor antagonist reported by Eisenberg, S., et al. (1990), Nature, 343:341; and Carter, D., et al (1990), Nature, 344:633.

Interleukin-1 receptor antagonist is a naturally occurring peptide secreted by macrophages in response to many of the same stimuli which cause the secretion of Interleukin-1 itself. Interleukin-1 receptor antagonist is a naturally occurring antagonist to the cytokines and recognizes receptors on various cell types and blocks Interleukin-1 mediated responses by occupying the receptor. (Wakabayashi et al., FASEB J 1991;5:338; Okusawa et al. J Clin Invest 1988;81:1162; Ohlsson et al., Nature 1990;348:550; Aiura, et al. Cytokine 1991;4:498; Fischer et al. Am J Physiol 1991;261:R442). In humans, Interleukin-1 receptor antagonist is a naturally occurring group of molecules; three forms have been characterized (two glycosylated and one non-glycosylated).

Fischer et al. (Am J Physiol 1991;261:R442) demonstrated that the administration of a naturally occurring antagonist to Interleukin-1 will significantly blunt the cytokine cascade and improve survival in baboons given a lethal dose of live bacteria. Interleukin-1 receptor antagonist significantly attenuates the decrease in mean arterial pressure and cardiac output and improves survival for severe acute pancreatitis. (U.S. Pat. NO. 5 ,508,262) The systemic Interleukin-1 response observed as a result of bacterial sepsis was also diminished significantly, correlating with a decrease in the systemic response to bacterial sepsis.

Studies by Aiura et al. (Cytokine 1991;4:498) have shown that Interleukin-1 receptor antagonist is protective in a rabbit model of hypotensive gram-positive septic

shock. The administration of Interleukin-1 receptor antagonist in this animal model has been shown to maintain mean arterial pressure compared to control, as well as decreasing lung water and maintaining urine output. This work demonstrated the role of Interleukin-1 and the protective role of Interleukin-1 receptor antagonist in gram-positive septic shock. Interleukin-1 is the principal mediator in a patient's clinical response to multiple different stresses regardless of the etiology (including acute pancreatitis, sepsis, endotoxin shock, and cytokine induced shock).

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising the amino acid sequence of SEQ ID NOS: 2, 4, or 13, or the amino acid sequence encoded by the cDNA insert of clone pIL-1Hy2, or a portion thereof corresponding to the full length or mature protein. Polypeptides of the invention also include polypeptides with IL-1 Hy2 activity that are encoded by (a) the polynucleotide of SEQ ID NOS: 1, 12 or 14, or (b) polynucleotides encoding SEQ ID NOS: 2, 4, or 13 (b) polynucleotides that hybridize to the complement of the polynucleotides of either (a) or (b) under stringent hybridization conditions. Biologically active or immunologically active variants of the IL-1Ra protein sequence of SEQ ID NOS: 2, 4 or 13 and "substantial equivalents" thereof (e.g., with 65%, 70%, 75%, 80%, 85%, 90%, typically 95%, more typically 98% or most typically 99% amino acid identity) that retain IL-1 Hy2 activity, preferably IL-1 antagonist activity, are also contemplated. Polypeptides encoded by allelic variants, such as those described in Example 2 below, may have a similar or increased or decreased activity compared to the polypeptides of SEQ ID NOS: 2, 4 or 13.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

The invention also relates to methods for producing a polypeptide comprising growing a culture of the cells of the invention in a suitable culture medium, and purifying the protein from the culture. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, and further purified. Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (e.g., an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs that encode proteins. A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography. See, e.g., Scopes, Protein Purification: Principles and Practice, Springer-Verlag (1994); Sambrook, et al., in Molecular Cloning: A Laboratory Manual; Ausubel et al., Current Protocols in Molecular Biology.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention. The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for e.g., small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are

then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the binding molecules may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for SEQ ID NOS: 2, 4 or 13.

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Pat. No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, Calif., U.S.A. (the MAXBAT™. kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, HEPARIN-TOYOPEARL™ or CIBACROM BLUE 3GA SEPHAROSE™; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and In Vitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The polypeptides of the invention include Interleukin-1 Hy2 analogs or variants. This embraces fragments of IL-1 Hy2 of the invention, as well as analogs (variants) of IL-1 Hy2 in which one or more amino acids has been deleted, inserted, or substituted. Analogs of the invention also embrace fusions or modifications of IL-1 Hy2 wherein the IL-1 Hy2 or analog is fused to another moiety or moieties, e.g., targeting moiety or another therapeutic agent. Such analogs may exhibit improved properties such as activity and/or stability. Examples of moieties which may be fused to IL-1 Hy2 or an analog include, for example, targeting moieties which provide for the delivery of polypeptide to pancreatic cells, e.g., antibodies to pancreatic cells, antibodies to immune cells such as T-cells, monocytes, dendritic cells, granulocytes, etc., as well as receptor and ligands expressed on pancreatic or immune cells. Other moieties which may be fused to IL-1 Hy2 or an analog include therapeutic agents which are used for treatment, for example, immunosuppressive drugs such as cyclosporin, SK506, azathioprine, CD3 antibodies and steroids, or immunostimulants, immune modulators, and other cytokines such as alpha or beta interferon.

5. DEPOSIT OF CLONE

The following clone, pIL-1Hy2 was deposited with the American Type Culture Collection (ATCC) 10801 University Avenue, Manassas, Virginia, on May 21, 1999 under the terms of the Budapest Treaty. The clone represents a plasmid clone as described in the Examples set forth below.

Microorganism/Clone	ATCC Accession No.
pIL-1Hy2	PTA-96

6. USES AND BIOLOGICAL ACTIVITY

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

6.1. RESEARCH USES AND UTILITIES

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine

levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

6.2. NUTRITIONAL USES

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

6.3. CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION ACTIVITY

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or

may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK. The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interleukin .gamma., Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6--Nordan, R. In

Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Aced. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--Bennett, F., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9--Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

6.4. IMMUNE STIMULATING OR SUPPRESSING ACTIVITY

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections

such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer. IL-1 has been indicated to promote tumor cell growth in cancers of various organs including breast adenocarcinoma, brain tumors, melanoma, myeloma, giant cell tumors of bone, acute myelogenous leukemia, oral epidermoid carcinoma, and squamous cell carcinoma; thus treatment of such cancer disease states involving elevated levels of IL-1 with IL-1 Hy2 polypeptides of the present invention is expected to ameliorate signs and symptoms of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein (or antagonists thereof, including antibodies) of the present invention may also be useful in the treatment of allergic reactions (*e.g.*, anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies) and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention. The therapeutic effects of IL-1 Hy2 polypeptides or antagonists thereof on allergic reactions can be evaluated by in vivo animals models such as the cumulative contact enhancement test (Lastbom et al., Toxicology 125: 59-66, 1998), skin prick test (Hoffmann et al., Allergy 54: 446-54, 1999), guinea pig skin sensitization test (Vohr et al., Arch. Toxocol. 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by

suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac

grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient mounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class II α chain protein and an MHC class II β chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.

M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., I. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264,

1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

6.5. HEMATOPOIESIS REGULATING ACTIVITY

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic

anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M. G. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I. K. and Briddell, R. A. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Long term culture initiating cell assay, Sutherland, H. J. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

6.6. TISSUE GROWTH ACTIVITY

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also

useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

6.7. ACTIVIN/INHIBIN ACTIVITY

A protein of the present invention may also exhibit activin- or inhibin-related activities. A polynucleotide of the invention may encode a polypeptide exhibiting such characteristics. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α-family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A protein of the invention may also be useful for

advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

6.8. CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement

and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Marguiles, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28); Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

6.9. HEMOSTATIC AND THROMBOLYTIC ACTIVITY

A protein of the invention may also exhibit hemostatic or thrombolytic activity. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

6.10. RECEPTOR/LIGAND ACTIVITY

A protein of the present invention may also demonstrate activity as receptors , receptor ligands or inhibitors or agonists of receptor/ligand interactions. A polynucleotide of the invention can encode a polypeptide exhibiting such characteristics. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and

receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

By way of example, the IL-1 Hy2 polypeptides of the invention may be used as a ligand for a cytokine receptor thereby modulating (*i.e.*, enhancing or inhibiting) the biological activity of that receptor. Examples of cytokine receptors that may be used include, but are not limited to, the Interleukin-1 Type I or Type II Receptors. Whether the IL-1 Hy2 polypeptides of the invention exhibit agonist, partial agonist, antagonist, or partial antagonist activity for a particular receptor, such as a cytokine receptor, in a particular cell type can be determined by conventional techniques known to those skilled in the art, such as those described below in sections 6.11.1 and 6.11.2 and in the Examples below. In one embodiment, one or more cells expressing a cytokine receptor (e.g., Interleukin-1 Type I or Type II Receptors) are contacted with the protein of the invention. Examples of cells that may be contacted with the protein of the invention include, but are not limited to, mammalian cells such as fibroblasts and T-cells. Preferably the novel protein of the invention acts as an antagonist for a cytokine receptor (e.g.-the Interleukin-I Receptor) so that the biological activities of that receptor are inhibited.

Studies characterizing drugs or proteins as agonist or antagonist or partial agonists a partial antagonist require the use of other proteins as competing ligands. The polypeptides of the present invention are expected to exhibit an affinity for Interleukin-1 Receptor. Thus, the polypeptides of the present invention may be used, for example, as competitors in assays involving Interleukin-1 Receptors. Alternatively, the polypeptides of the invention may be labelled by being coupled to radioisotopes, colorimetric molecules or a toxin molecules by conventional methods. ("Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic Press, Inc. San Diego) and used in both in vivo and in vitro to bind to the Interleukin-1 Receptor. Examples of radioisotopes include, but are not limited to, tritium and carbon-14 . Examples of colorimetric molecules include, but are not limited to, fluorescent molecules such as fluorescamine, or rhodamine or other colorimetric molecules. Examples of toxins include, but are not limited, to ricin. By way of example, the proteins coupled to such molecules are useful in studies involving in vivo or in vitro metabolism of the Interleukin-1 Receptor.

6.11 DRUG SCREENING WITH INTERLEUKIN-1 Hy2 POLYPEPTIDES

This invention is particularly useful for screening compounds by using the IL-1 Hy2 polypeptides of the invention, particularly binding fragments, in any of a variety of drug screening techniques. The polypeptides employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the desired IL-1 Hy2 polypeptide. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between IL-1 Hy2 polypeptides of the invention and the agent being tested or examine the diminution in complex formation between the IL-1 Hy2 polypeptides and an appropriate cell line, which are well known in the art.

6.11.1 ASSAY FOR ANTI-INTERLEUKIN-1 RECEPTOR ACTIVITY

In one embodiment, the Interleukin-1 receptor antagonist activity of the polypeptides of the invention is determined using a method that involve (1) forming a mixture comprising Interleukin-1, the Interleukin-1 receptor, and the IL-1 Hy2 polypeptides of the invention and/or its agonists and antagonists (or agonist or antagonist drug candidates) and/or antibodies specific for the IL-1 Hy2 polypeptides of the invention; (2) incubating the mixture under conditions whereby, but for the presence of said IL-1 Hy2 polypeptide of the invention and/or its agonists and antagonists (or agonist or antagonist drug candidates) and/or antibodies specific for the IL-1 Hy2 polypeptides of the invention, the Interleukin-1 binds to the Interleukin-1 receptor; and (3) detecting the presence or absence of specific binding of Interleukin-1 to the Interleukin-1 receptor.

6.11.2 ASSAY FOR ANTAGONISTS AND AGONISTS

Human HepG2 cells are incubated at 37 degree(s) C. for 18-24 hours in serum-free Dulbecco's modified Eagle medium. Separate monolayers of cells are incubated in the same medium supplemented with Interleukin-1 at various concentrations and in the same medium supplemented with a IL-1 Hy2 polypeptide of the invention at various concentrations.

Monolayers are rinsed vigorously with isotonic buffer and incubated in (35-S) methionine, 250 mu ci/ml methionine-free medium and pulsed for a period of 15-30 minutes to assess net synthesis. Cell culture fluid is discarded and monolayers are again rinsed and resuspended in cell lysis buffer. The newly synthesized radiolabelled hepatic proteins in these cell lysates are detected by immunoprecipitation, SDS-PAGE and fluorography.

6.12. ANTI-INFLAMMATORY ACTIVITY

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an

inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation intimation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material. In particular, the IL-1 Hy2 polypeptides of this invention may be utilized to prevent or treat condition such as, but not limited to, utilized, for example, as part of methods for the prevention and/or treatment of disorders involving sepsis, acute pancreatitis, endotoxic shock, cytokine induced shock, rheumatoid arthritis, chronic inflammatory arthritis, pancreatic cell damage from diabetes mellitus type 1, graft versus host disease, inflammatory bowel disease, inflamation associated with pulmonary disease, other autoimmune disease or inflammatory disease, an antiproliferative agent such as for acute or chronic mylegenous leukemia or in the prevention of premature labor secondary to intrauterine infections.

6.13 MODULATION OF IL-18, IL-12 and IFN-γ RELATED DISORDERS

Administration of IL-1Hy2 polynucleotides, polypeptides and agonists is also expected to be useful for the treatment of IL-18 and/or IL-12 and/or IFN-γ related disorders. IL-1Hy2 inhibits IL-18 and IL-12 activity, including IL-18 and IL-12 induced IFN-γ production.

IL-18 has been found to have a variety of biological activities including the stimulation of activated T cell proliferation, enhancement of NK cell lytic activity, induction of IFNγ secretion, enhancement of Fas ligand expression and function, and stimulation of granulocyte-macrophage colony-stimulating factor (GM-CSF) production by activated T cells. IL-18 has been shown to counteract viral and intracellular infections and suppress tumor formation. However, IL-18 is also involved in the pathogenic progression of chronic inflammatory diseases, including endotoxin-induced shock, liver injury (including endotoxin-induced liver injury, hepatitis, biliary atresia and obesity-related fatty liver) and autoimmune diseases. Other disorders related to IL-18 production

include meliodosis, purine nucleoside phosphorylase deficiency, increased susceptibility to Leishmania major and Staphylococcus aureus infection, hemophagocytic lymphohistiocytosis, mononucleosis, viral meningitis/encephalitis, bacterial meningitis/encephalitis and ischemia or ischemia/reperfusion injury.

Inflammation may result from infection with pathogenic organisms (including gram-positive bacteria, gram-negative bacteria, viruses, fungi, and parasites such as protozoa and helminths), transplant rejection (including rejection of solid organs such as kidney, liver, heart, lung or cornea, as well as rejection of bone marrow transplants including graft versus host disease (GVHD)), or from localized chronic or acute autoimmune or allergic reactions. Autoimmune diseases include acute glomerulonephritis; rheumatoid or reactive arthritis; chronic glomerulonephritis; inflammatory bowel diseases such as Crohn's disease, ulcerative colitis and necrotizing enterocolitis; granulocyte transfusion associated syndromes; inflammatory dermatoses such as contact dermatitis, atopic dermatitis, psoriasis; systemic lupus erythematosus (SLE), autoimmune thyroiditis, multiple sclerosis, some forms of diabetes, or any other autoimmune state where attack by the subject's own immune system results in pathologic tissue destruction. Allergic reactions include allergic asthma, chronic bronchitis, allergic rhinitis, acute and delayed hypersensitivity. Systemic inflammatory disease states include inflammation associated with trauma, burns, reperfusion following ischemic events (e.g. thrombotic events in heart, brain, intestines or peripheral vasculature, including myocardial infarction and stroke), sepsis, ARDS or multiple organ dysfunction syndrome. Inflammatory cell recruitment also occurs in atherosclerotic plaques.

Endotoxin activation of the systemic inflammatory response leads to a number of disorders including bacterial and/or endotoxin-related shock, fever, tachycardia, tachypnea, cytokine overstimulation, increased vascular permeability, hypotension, complement activation, disseminated intravascular coagulation, anemia, thrombocytopenia, leukopenia, pulmonary edema, adult respiratory distress syndrome, intestinal ischemia, renal insufficiency and failure, and metabolic acidosis.

Hepatitis represents liver disorders that are characterized by hepatic inflammation and necrosis that can be manifested as an acute or chronic condition. These liver disorders include virus-induced hepatitis such as hepatitis A, hepatitis B, hepatitis C (non-A, non-B hepatitis), hepatitis D, hepatitis E; toxin and drug induced hepatitis such as

acetaminophohen hepatotoxicity, halothane hepatotoxicity, mehtyldopa hepatoxicity, iaoniazid hepatoxicity, sodium valproate hepatoxicity, phenytion hepatoxicity, chlorpromazine hepatoxicity, amiodarone hepatoxicity, amioidarone hepatoxicity, erythromycin hepatoxicity, oral contraceptive hepatoxicity, 17,α-alkyl-substituted anabolic steroid hepatoxicity and trimethoprim-sulfamethoxazole hepatoxicity; cholestatic hepatitis; alcoholic hepatitis; autoimmune chronic active hepatitis; and T cell mediated hepatitis. Other conditions that cause liver injury include congenital bilary atresia, obesity-related fatty liver and the autosomal recessive disease heamophagocytic lymphohistocytosis (HLH).

IL-18 induced IFN-γ plays a role in liver injury. IFNγ has been shown to mediate LPS-induced liver injury following Propionibacterium acnes infection as described in Tsuji et al. (J. Immunol. 162: 1049-55, 1999). Large number of macrophages and lymphocytes infiltrate the portal area in response to P. acnes infection which results in intrahepatic formation of granulomas. IFNγ knock out mice exhibited less macrophage infiltration and a reduction in the number and size of granulomas. Subsequent treatment with low doses of LPS caused massive hepatic necrosis and increased IL-12, IL-18 and TNF-α serum levels in the normal mice, while the knock out mice exhibited drastic decreases in IL-12, IL-18 and TNF-α serum levels. The addition of IFNγ neutralizing antibody also caused a decrease in IL-18 and IL-12 levels. This model of liver injury indicates that LPS-induced liver injury is associated with increased levels of IL-18, IL-12 and IFN-γ. Currently, a role for IL-1β is not known in this liver injury model. Since IL-1β is known to be induced by LPS, it is possible IL-1β also plays a role in the disorder. Treatment with IL-Ra may modulate the severity of liver injury due to IL-18 induced IFN-γ production and IL-1β.

IL-18 has also been shown to be involved in the immunomediated hepatitis model where treatment with concavalin A induced hepatitis in mice as described by Fiorucci et al. (Gastroenterology 118: 404-21, 2000). In this model, CD+ Tcells and Th1-like cytokines cause Fas mediated liver cell death. Treatment with a nitric oxide derivative of aspirin protected against this cell death by reducing production of IFNγ, IL-18, IL-12, IL-1β and TNF-α. In addition, a neutralizing antibody to IL-18 caused a decrease in IFNγ production and reduced liver injury induced by conA.

HLH is a fatal autosomal recessive disease that manifests in early childhood. This disease is characterized by fever, hepatosplenomegaly, cytopenia and widespread infiltration of vital organs by activated lymphocytes and macrophages. Patients with HLH exhibit elevated serum levels of IL-18. IL-18 plays an important role in the induction of Th1 cells in HLH patients. (Takada et al., Br. J. Haematol. 106: 182-9, 1999).

IL-1 Hy2 inhibits IL-18 induced production of IFNγ. In the models described above, the degree of IL-1β activity is not known. Since IL-1β is known to be induced by LPS, it is possible that IL-1β also play a role in the pathogenicity of these conditions. The presence of the appropriate amount of IL-1Hy2 polynucleotides, polypeptides or other agonists may modulate the severity of the disease states due to both IL-18 induced IFNγ production and IL-1β.

IL-12 is known to potentiate IFNγ production, and the cytolytic activity of NK cells and cytotoxic T lymphocytes. These immunomodulatory effects have implicated a role for IL-12 in therapies for cancer and infectious disease. However, these same therapeutic effects can also promote autoimmune diseases and chronic inflammatory conditions such as multiple sclerosis, transplant rejection and cytotoxicity.

IL-12 and IFN-γ are involved in the pathogenesis of multiple sclerosis (MS). In the experimental allergic encephalomyelitis animal model (EAE), the demyelinating effect on the central nervous system is carried out similar to that in humans suffering from MS. Currently, IFNβ is used to treat MS. The mechanism of IFNβ treatment may be to decrease the number of IFNγ producing T cells in MS patients. (Rep et al., J. Neuroimmunol. 96:92-100, 1999). In addition, IFNγ production in blood lymphocytes was found to correlate with disability score in MS patients. (Petcreit et al., Mult. Scler. 6: 19-23, 2000). Antibodies against IL-12 were found to prevent superantigen-induced and spontaneous relapses of EAE in mice (Constantineseu et al., J. Immunol. 161: 5097-5104, 1998). All these studies point to the involvement of IL-12 induced IFNγ production in the progression of MS in human patients. Therefore, treatment with IL-1 Hy2 polynucleotides, polypeptides or other agonists to reduce IFNγ production may be an useful therapy for MS patients.

The combination of IL-12 and IL-2 has synergistic anti-tumor activity in vivo. However, in clinical trials the combination resulted in significant toxicity and

subsequently shock and mortality. (Cohen, Science 270: 908 1995). In a murine model investigated by Carson et al. (J. Immunol., 162: 4943-5, 1999) determined that the fatal systemic inflammatory response was NK cell dependent but not related to other effector molecules in the system such as IL-1, TNF-α, and IFNγ. IL-1 Hy2 polynucleotides, polypeptides or other agonists is expected to inhibit IL-12 induced IFN-γ production and is expected to inhibit other biological activities of IL-12 such as NK cell cytolytic activity. Inhibition of NK cell activity, through IL-Ra administration, may reduce toxicity resulting from IL-12 antitumor treatment.

The effect of IL-1 Hy2 on IL-12 and/or IL-18 activity may be determined by measuring the biological activities of these cytokines. Both IL-12 and IL-18 are known to induce IFNγ production in T cells. In addition to IFN-γ, the combination of IL-12 and IL-18 increases production of IL-3, IL-6 and TNF. Treatment with IL-1 Hy2 is expected to reduce IFNγ production induced by IL-12 and IL-18. Circulating or local levels of IFNγ in tissue or fluid samples from patients treated with IL-1 Hy2 polynucleotides, polypeptides or other agonists will be an indication of the therapeutic effects of IL-1 Hy2 on the IL-18 and IL-12 related disorders. Tissue samples include tissue samples from an area involved in inflammation or other disease. Fluid samples include, for example, whole blood, plasma, serum, cerebrospinal fluid, synovial fluid, peritoneal fluids (including lavage fluids or exudate), pleural fluids (including lavage fluids or exudate), wound fluids (including lavage fluids or exudate).

Furthermore, IL-12 is known to activate NK cells and to decrease serum IgE levels. These assays may also be used to measure the effectiveness of IL-1 Hy2 treatment for IL-12 related disorders. The NK cell cytolytic activity in patients treated with IL-1 Hy2 polynucleotides, polypeptides or other agonists can be assayed by measuring patient's blood samples ability to lysis colon carcinoma or lymphoma cells in vitro. (Lieberman et al., J. Sur. Res., 50: 410-415, 1992) In addition, the serum levels of IgE from patients treated with IL-1Hy2 can be measured to determine the effectiveness of treatment for IL-12 related disorders. (Kiniwa et al. J. Clin. Invest., 90 : 262-66, 1992)

6.14. LEUKEMIAS

Leukemias and related disorders may be treated or prevented by administration of a therapeutic that promotes or inhibits function of the polynucleotides and/or polypeptides

of the invention. Such leukemias and related disorders include but are not limited to acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia).

6.15. NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested for efficacy of intervention with compounds that modulate the activity of the polynucleotides and/or polypeptides of the invention, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

(i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;

(ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;

(iii) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;

(iv) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

(v) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;

(vi) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;

(vii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

(viii) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, therapeutics which elicit any of the following effects may be useful according to the invention:

(i) increased survival time of neurons in culture;

(ii) increased sprouting of neurons in culture or in vivo;

(iii) increased production of a neuron-associated molecule in culture or in vivo, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

(iv) decreased symptoms of neuron dysfunction in vivo.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction

may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In a specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

6.16. OTHER ACTIVITIES

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, co-factors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an

immune response against such protein or another material or entity which is cross-reactive with such protein.

6.17 IDENTIFICATION OF POLYMORPHISMS

The demonstration of polymorphisms, for example the T125C, C184T and A205C polymorphisms illustrated in Example 2 below, makes possible the identification of such polymorphisms in human subjects and the pharmacogenetic use of this information for diagnosis and treatment. Such polymorphisms may be associated with, e.g., differential predisposition or susceptibility to various disease states (such as disorders involving inflammation or immune response) or a differential response to drug administration, and this genetic information can be used to tailor preventive or therapeutic treatment appropriately. For example, the existence of a polymorphism associated with a predisposition to inflammation or autoimmune disease makes possible the diagnosis of this condition in humans by identifying the presence of the polymorphism.

Polymorphisms can be identified in a variety of ways known in the art which all generally involve obtaining a sample from a patient, analyzing DNA from the sample, optionally involving isolation or amplification of the DNA, and identifying the presence of the polymorphism in the DNA. For example, PCR may be used to amplify an appropriate fragment of genomic DNA which may then be sequenced. Alternatively, the DNA may be subjected to allele-specific oligonucleotide hybridization (in which appropriate oligonucleotides are hybridized to the DNA under conditions permitting detection of a single base mismatch) or to a single nucleotide extension assay (in which an oligonucleotide that hybridizes immediately adjacent to the position of the polymorphism is extended with one or more labelled nucleotides). In addition, traditional restriction fragment length polymorphism analysis (using restriction enzymes that provide differential digestion of the genomic DNA depending on the presence or absence of the polymorphism) may be performed.

Alternatively a polymorphism resulting in a change in the amino acid sequence could also be detected by detecting a corresponding change in amino acid sequence of the protein, e.g., by an antibody specific to the variant sequence.

7. THERAPEUTIC METHODS

The novel IL-1 Hy2 polypeptides (including fragments, analogs and variants) of the invention have numerous applications in a variety of therapeutic methods. Antagonists and agonist of IL-1 Hy2 polypeptides may also have therapeutic applications in these models. Examples of therapeutic applications include, but are not limited to, those exemplified below.

7.1 SEPSIS

One embodiment of the invention is the administration of an effective amount of the IL-1 Hy2 polypeptides of the invention or modulators of IL-1 Hy2 polypeptides (such as agonists or antagonists) to individuals that are at a high risk of developing sepsis, or that have developed sepsis. An example of the former category are patients about to undergo surgery. While the mode of administration is not particularly important, parenteral administration is preferred because of the rapid progression of sepsis, and thus, the need to have the inhibitor disseminate quickly throughout the body. Thus, the preferred mode of administration is to deliver an I.V. bolus slightly before, during, or after surgery. The dosage of the IL-1 Hy2 polypeptides of the invention or IL-1 Hy2 modulators will normally be determined by the prescribing physician. It is to be expected that the dosage will vary according to the age, weight and response of the individual patient. Typically, the amount of inhibitor administered per dose will be in the range of about 0.1 to 25 mg/kg of body weight, with the preferred dose being about 0.1 to 10 mg/kg of patient body weight. For parenteral administration, the IL-1 Hy2 polypeptides of the invention or IL-1 Hy2 modulators will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of additives that maintain the isotonicity and stability of the inhibitor. The preparation of such solutions is within the skill of the art. Typically, the cytokine inhibitor will be formulated in such vehicles at a concentration of about 1-8 mg/ml to about 10 mg/ml.

7.2 ARTHRITIS AND INFLAMMATION

The immunosuppressive effects of the Interleukin-1 inhibitor against rheumatoid arthritis is determined in an experimental animal model system. The experimental model system is adjuvant induced arthritis in rats, and the protocol is described by J. Holoshitz, et at., 1983, Science, 219:56, or by B. Waksman et al., 1963, Int. Arch. Allergy Appl. Immunol., 23:129. Induction of the disease can be caused by a single injection, generally intradermally, of a suspension of killed Mycobacterium tuberculosis in complete Freund's adjuvant (CFA). The route of injection can vary, but rats may be injected at the base of the tail with an adjuvant mixture. The inhibitor is administered in phosphate buffered solution (PBS) at a dose of about 1-5 mg/kg. The control consists of administering PBS only.

The procedure for testing the effects of the Interleukin-1 inhibitor would consist of intradermally injecting killed Mycobacterium tuberculosis in CFA followed by immediately administering the inhibitor and subsequent treatment every other day until day 24. At 14, 15, 18, 20, 22, and 24 days after injection of Mycobacterium CFA, an overall arthritis score may be obtained as described by J. Holoskitz above. An analysis of the data would reveal that the inhibitor would have a dramatic affect on the swelling of the joints as measured by a decrease of the arthritis score.

7.3 DIABETES

Interleukin-1 has been shown to be involved in the destruction of islet cells in diabetes mellitus (DM) (Mandrup-Paulsen, T., K. Bendtzen, J. Nerup, C. A. Dinarello, M. Svenson, and J. H. Nielson [1986] Diabetologia 29:63-67). The IL-1 Hy2 polypeptides of the invention limit lymphocyte and macrophage mediated damage to islet cells in incipient cases of DM identified by disease susceptibility via genetic background and family history. The inflammatory destruction of the pancreatic beta islet cells in such individuals with early DM is reduced by parenterally administering the IL-1 Hy2 polypeptides of the invention or modulators of IL-1 Hy2 polypeptides (such as agonists or antagonists) which have an anti-Interleukin-1 effect in the pancreas.

7.4 ANTI-HYPOTENSIVE ARGININE-FREE FORMULATIONS

The parenteral formulation of the therapeutic regimen is defined as including: about 3-4 g/l isoleucine, about 4-6 g/l leucine, about 3-4 g/l lysine, about 1-2 g/l methionine, about 1-2 g/l phenylalanine, about 2-3 g/l threonine, about 0.5-1.5 g/l tryptophan, about 3-4 g/l valine, about 4-5 g/l alanine, about 1-2 g/l histidine, about 3-4 g/l proline, about 1-2 g/l serine, about 0.25-0.75 g/l tyrosine, about 4-5 g/l glycine and about 2-3 g/l aspartic acid, together in a pharmacologically acceptable excipient. In another preferred embodiment of the described parenteral formulation, the formulation may further include ornithine, most particularly at a concentration of about 1-2 g/1. In still another embodiment of the described parenteral formulation, the formulation may include citrulline, most preferably at a concentration of between about 1 g/l and about 2 g/l. Both citrulline and ornithine may be included in still another embodiment of the formulation, again at the concentrations indicated.

The method includes an arginine-free formulation which comprises the amino acids and concentrations thereof already described herein, together in a pharmacologically acceptable excipient. Again, the formulation may further include ornithine, citrulline, or both, to even further supply physiologically required concentrations of urea cycle substrates in the animal. Most preferably, the formulation is provided as a parenteral formulation.

Another aspect of the method comprises a method for treating chemotherapeutic agent-related hypotension. In a most preferred embodiment, the method comprises monitoring an animal receiving a chemotherapeutic agent for a decrease in systolic blood pressure to less than about 100 mm Hg to detect an animal with systemic hypotension, treating the animal having systemic hypotension with a therapeutic regimen comprising a therapeutically effective amount of an arginine-free formulation sufficient to reduce plasma or serum arginine concentrations administered concurrently with or followed by the administration of a therapeutically effective concentration of an IL-1 Hy2 polypeptide or modulators of IL-1 Hy2 polypeptides (such as agonists or antagonists) , and maintaining the animal on the therapeutic regimen until an increase of systolic blood pressure to at least about 100 mm Hg is detectable. Most preferably, the arginine-free formulation is a parenteral formulation.

In a preferred embodiment, the IL-1 Hy2 polypeptides of the invention or modulators of IL-1 Hy2 polypeptides (such as agonists or antagonists) are used in combination with the anti-hypotensive arginine free formulation to treat hypotension in an animal, particularly that hypotension caused by exposure to endotoxin or septic shock.

A patient having a systolic blood pressure of less than about 100 mm Hg will be targeted for the present treatment. Such a patient is to be placed on a continuous feed of an arginine-free formulation which includes a mixture of essential and nonessential amino acids as described in U.S. Patent NO. 5,334,380. The patient is treated concurrently with the interleukin-1 antagonist polypeptides of the invention. Blood samples are to be obtained from the patient and arginine levels in the serum or plasma fraction are determined.

7.5 PHARMACEUTICAL FORMULATIONS AND ROUTES OF ADMINISTRATION

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin.

The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Protein that can be administered with IL-1 Hy2 include other IL-1 receptor antagonist

polypeptides such as IL-1Ra and IL-1 Hy1 Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent. A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, e.g., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

7.6. ROUTES OF ADMINISTRATION

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops.Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

7.7. COMPOSITIONS/FORMULATIONS

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably

from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose,

sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative.

The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its

solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Many of the proteinase inhibiting compounds of the invention may be provided as salts with pharmaceutically compatible counterions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins

including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention. The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 μg to about 100 mg (preferably about 0.1 μg to about 10 mg, more preferably about 0.1 μg to about 1 mg) of protein of the present invention per kg body weight. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery

to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being

cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorbtion of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-.alpha. and TGF-.beta.), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention. The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either in vivo or ex vivo into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured

ex vivo in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes.

7.8. EFFECTIVE DOSAGE

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the C-proteinase activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, e.g., Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1.Dosage amount and

interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the C-proteinase inhibiting effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from in vitro data; for example, the concentration necessary to achieve 50-90% inhibition of the C-proteinase using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

An exemplary dosage regimen for the human IL-1 Hy2 polypeptides of the invention will be in the range of about 0.01 to 100 mg/kg of body weight daily, with the preferred dose being about 0.1 to 25 mg/kg of patient body weight daily, varying in adults and children. Dosing may be once daily, or equivalent doses may be delivered at longer or shorter intervals.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's age and weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

7.9. PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition.

8. ANTIBODIES

Another aspect of the invention is an antibody that specifically binds the polypeptide of the invention. Such antibodies can be either monoclonal or polyclonal antibodies, as well fragments thereof and humanized forms or fully human forms, such as those produced in transgenic animals. The invention further provides a hybridoma that produces an antibody according to the invention. Antibodies of the invention are useful for detection and/or purification of the polypeptides of the invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R. P. Merrifield, J. Amer. Chem. Soc. 85, 2149-2154 (1963); J. L. Krstenansky, et al., FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein. In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A.M., Monoclonal Antibodies Technology: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth et al., J. Immunol. 35:1-21 (1990); Kohler and Milstein, Nature 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today 4:72 (1983); Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985), pp. 77-96).

Any animal (mouse, rabbit, etc.) which is known to produce antibodies can be immunized with a peptide or polypeptide of the invention. Methods for immunization are well known in the art. Such methods include subcutaneous or intraperitoneal injection of

the polypeptide. One skilled in the art will recognize that the amount of the protein encoded by the ORF of the present invention used for immunization will vary based on the animal which is immunized, the antigenicity of the peptide and the site of injection. The protein that is used as an immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to, coupling the antigen with a heterologous protein (such as globulin or β-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz et al., Exp. Cell Research. 175:109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)). Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to proteins of the present invention.

For polyclonal antibodies, antibody containing antiserum is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures. The present invention further provides the above-described antibodies in delectably labeled form. Antibodies can be delectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.) fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labeling are well-known in the art, for example, see (Sternberger, L.A. et al., J. Histochem. Cytochem. 18:315 (1970); Bayer, E.A. et al., Meth. Enzym. 62:308 (1979); Engval, E. et al., Immunol. 109:129 (1972); Goding, J.W. J. Immunol. Meth. 13:215 (1976)).

The labeled antibodies of the present invention can be used for in vitro, in vivo, and in situ assays to identify cells or tissues in which a fragment of the polypeptide of interest is expressed. The antibodies may also be used directly in therapies or other diagnostics.The present invention further provides the above-described antibodies immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir, D.M. et al., "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby, W.D. et al., Meth. Enzym. 34 Academic Press. N.Y. (1974)). The immobilized antibodies of the present invention can be used for in vitro, in vivo, and in situ assays as well as for immuno-affinity purification of the proteins of the present invention.

The three-dimensional structural analysis of IL-1 Hy2 (described in Example 14) demonstrates the IL-1 Hy2 residues involved in IL-1 receptor interactions. Antibodies that specifically bind to these receptor interacting residues are preferred antagonists for IL-1 Hy2 activity. These antibodies will reduce IL-1 Hy2 binding to an IL-1 receptor and thereby inhibit IL-1 Hy2 activity.

9. COMPUTER READABLE SEQUENCES AND STRUCTURAL COORDINATES

According to one aspect of this invention, a nucleotide sequence, amino acid sequence or three-dimensional structure of the present invention can be recorded on computer readable media. A three-dimensional structure may be represented or displayed using structural coordinates of atoms of amino acids within amino acid sequences of the present invention (including mutant or variant amino acid sequences), particularly amino acids involved in binding to IL-1 receptor or other receptors or IL-1 receptor accessory protein, as well as amino acids involved in other IL-1Hy2 functions.

As used herein, "computer readable media" or "machine readable storage medium" refers to any medium which can be read and accessed directly by a computer. The term "data storage material" refers to any material on which data can be physically stored. Such media include, but are not limited to: magnetic storage media, such as

floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. The term "machine readable data" refers to a group of one or more characters, including numbers, representing basic elements of information that can be processed by a computer. A skilled artisan can readily appreciate how any of the presently known computer readable media can be used to create a manufacture comprising a computer readable medium having recorded thereon a nucleotide sequence, amino acid sequence or structural coordinates of the present invention that can be used to render a three-dimensional structure of a polypeptide.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the sequence or structure information of the present invention. A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon sequence or structure information of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the sequence or structure information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of dataprocessor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the sequence or structure information of the present invention.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. As stated above, the computer-based systems of the present invention comprise a data

storage means having stored therein sequence or structure information of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store sequence or structure information of the present invention, or a memory access means which can access manufactures having recorded thereon the sequence or structure information of the present invention.

Input means can be implemented in a variety of ways. Machine-readable data of this invention may be inputted via the use of a modem or modems connected by a telephone line or dedicated data line. Alternatively or additionally, the input means may comprise CD-ROM drives or disk drives. In conjunction with a display terminal, a keyboard may also be used as an input device. Output means may similarly be implemented by conventional devices. By way of example, output hardware may include CRT display terminal for displaying a graphical representation of important functional residues of the invention using a computer program as described herein. Output means might also include a printer, so that hard copy output may be produced, or a disk drive to store system output for later use.

In operation, the CPU coordinates the use of the various input and output devices, coordinates data accesses from data storage means including working memory, and determines the sequence of data processing steps. A number of programs may be used to process the machine-readable data of the invention, to form or display a sequence or a three-dimensional structure or representation, or to carry out computational methods of sequence comparison or drug discovery.

For example, by providing the nucleotide sequence of SEQ ID NOS: 1, 12 or 14 or a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NOS: 1, 12 or 14 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) and BLAZE (Brutlag et al., Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system is used to identify open reading frames (ORFs) within a nucleic acid sequence. Such ORFs may be protein encoding fragments and may be useful in producing

commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of a known sequence which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, MacPattern (EMBL), BLASTN and BLASTA (NPOLYPEPTIDEIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems. As used herein, a "target sequence" can be any nucleic acid or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that searches for commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

Computational methods of drug discovery may include computational evaluation of a three-dimensional structure for its ability to associate with moieties of chemical compounds. This evaluation may include performing a fitting operation between the structure or a portion thereof and one or more moieties of a chemical compound, and

thereby qualitatively or quantitatively judging the proximity and/or extent of interaction between the three-dimensional structure and the chemical moiety(ies). Interaction may take place through, e.g., non-covalent interactions such as hydrogen bonding, van der Waals interactions, hydrophobic interactions and electrostatic interactions, or through covalent bonding. When the structure is displayed in a graphical three-dimensional representation on a computer screen, this allows visual inspection of the structure, as well as visual inspection of the structure's association with chemical moieties.

Specialized computer programs may be used to assist in a process of selecting chemical moieties or fragments of chemical compounds for further evaluation. These include: 1. GRID (P. J. Goodford, "A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules", J. Med. Chem., 28, pp. 849-857 (1985)). GRID is available from Oxford University, Oxford, UK. 2. MCSS (A. Miranker et al., "Functionality Maps of Binding Sites: A Multiple Copy Simultaneous Search Method." Proteins: Structure, Function and Genetics, 11, pp. 29-34 (1991)). MCSS is available from Molecular Simulations, San Diego, Calif. 3. AUTODOCK (D. S. Goodsell et al., "Automated Docking of Substrates to Proteins by Simulated Annealing", Proteins: Structure, Function, and Genetics, 8, pp. 195-202 (1990)). AUTODOCK is available from Scripps Research Institute, La Jolla, Calif. 4. DOCK (I. D. Kuntz et al., "A Geometric Approach to Macromolecule-Ligand Interactions", J. Mol. Biol., 161, pp. 269-288 (1982)). DOCK is available from University of California, San Francisco, Calif.

Assembly of individual chemical moieties or fragments can be assisted by using programs including: 1. CAVEAT (P. A. Bartlett et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", in Molecular Recognition in Chemical and Biological Problems", Special Pub., Royal Chem. Soc., 78, pp. 182-196 (1989); G. Lauri and P. A. Bartlett, "CAVEAT: a Program to Facilitate the Design of Organic Molecules", J. Comput. Aided Mol. Des., 8, pp. 51-66 (1994)). CAVEAT is available from the University of California, Berkeley, Calif. 2. 3D Database systems such as ISIS (MDL Information Systems, San Leandro, Calif.). This area is reviewed in Y. C. Martin, "3D Database Searching in Drug Design", J. Med. Chem., 35, pp. 2145-2154 (1992). 3. HOOK (M. B. Eisen et al, "HOOK: A Program for Finding Novel Molecular Architectures that Satisfy the Chemical and Steric Requirements of a

Macromolecule Binding Site", Proteins: Struct., Funct., Genet., 19, pp. 199-221 (1994). HOOK is available from Molecular Simulations, San Diego, Calif.

Computer programs that assist in designing a chemical compound that potentially interacts with a three-dimensional structure as a whole or "de novo" using either an empty binding site or optionally including some portion(s) of a known modulator(s) include: 1. LUDI (H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", J. Comp. Aid. Molec. Design, 6, pp. 61-78 (1992)). LUDI is available from Molecular Simulations Incorporated, San Diego, Calif. 2. LEGEND (Y. Nishibata et al., Tetrahedron, 47, p. 8985 (1991)). LEGEND is available from Molecular Simulations Incorporated, San Diego, Calif. 3. LeapFrog (available from Tripos Associates, St. Louis, Mo.). 4. SPROUT (V. Gillet et al, "SPROUT: A Program for Structure Generation)", J. Comput. Aided Mol. Design, 7, pp. 127-153 (1993)). SPROUT is available from the University of Leeds, UK.

Other molecular modeling techniques may also be employed in accordance with this invention [see, e.g., N. C. Cohen et al., "Molecular Modeling Software and Methods for Medicinal Chemistry", J. Med. Chem., 33, pp. 883-894 (1990); see also, M. A. Navia and M. A. Murcko, "The Use of Structural Information in Drug Design", Current Opinions in Structural Biology, 2, pp. 202-210 (1992); L. M. Balbes et al., "A Perspective of Modern Methods in Computer-Aided Drug Design", in Reviews in Computational Chemistry, Vol. 5, K. B. Lipkowitz and D. B. Boyd, Eds., VCH, New York, pp. 337-380 (1994); see also, W. C. Guida, "Software For Structure-Based Drug Design", Curr. Opin. Struct. Biology,, 4, pp. 777-781 (1994)].

Binding affinity may be tested and optimized by computational evaluation, e.g. by minimizing the energy between the bound and free states of the three-dimensional structure (e.g., a small deformation energy of binding, preferably not greater than about 10 kcal/mole and more preferably not greater than 7 kcal/mole).

Specific computer software is available in the art to evaluate compound deformation energy and electrostatic interactions. Examples of programs designed for such uses include: Gaussian 94, revision C (M. J. Frisch, Gaussian, Inc., Pittsburgh, Pa.); AMBER, version 4.1 (P. A. Kollman, University of California at San Francisco); QUANTA/CHARMM (Molecular Simulations, Inc., San Diego, Calif.); Insight II/Discover (Molecular Simulations, Inc., San Diego, Calif.); DelPhi (Molecular

Simulations, Inc., San Diego, Calif.); and AMSOL (Quantum Chemistry Program Exchange, Indiana University). These programs may be implemented, for instance, using a Silicon Graphics workstation with "IMPACT" graphics. Other hardware systems and software packages will be known to those skilled in the art.

Such computational drug design may include computer-based screening of small molecule databases for chemical moieties or chemical compounds that can bind in whole, or in part, to the desired three-dimensional structure. In this screening, the quality of fit of such entities to the binding site may be judged either by shape complementarity or by estimated interaction energy [E. C. Meng et al., J. Comp. Chem., 13, pp. 505-524 (1992)].

10. TRIPLE HELIX FORMATION

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 15241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Olmno, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

11. DIAGNOSTIC ASSAYS AND KITS

The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using a nucleic acid probe or antibodies of the present invention.

In general, methods for detecting a polynucleotide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the

polynucleotide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polynucleotide of the invention is detected in the sample. Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polypeptide of the invention is detected in the sample. In detail, such methods comprise incubating a test sample with one or more of the antibodies or one or more of nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the nucleic acid probe or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention. Examples of such assays can be found in Chard, T., An Introduction to Radioimmunoassay and Related Techniques, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., Techniques in Immunocytochemistry, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1985). The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the probes or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound probe or antibody.

In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or probe. Types of detection reagents include labeled nucleic acid probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily recognize that the disclosed probes and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

12. MEDICAL IMAGING

The novel IL-1 Hy2 polypeptides of the invention are useful in medical imaging, e.g., imaging the site of infection, inflammation, and other sites having Interleukin-1 receptor antagonist receptor molecules. See, e.g., Kunkel et al., U.S. Pat. NO. 5,413,778. Such methods involve chemical attachment of a labelling agent, administration of the labelled IL-1 Hy2 polypeptide to a subject in a pharmaceutically acceptable carrier, and imaging the labelled IL-1 Hy2 polypeptide *in vivo* at the target site.

13. SCREENING ASSAYS

Using the isolated proteins and polynucleotides of the invention, the present invention further provides methods of obtaining and identifying agents which bind to a polypeptide encoded by the ORF from a polynucleotide with a sequence of SEQ ID NOS: 1, 12 or 14 to a specific domain of the polypeptide encoded by the nucleic acid, or to a nucleic acid with a sequence of SEQ ID NOS: 1, 12 or 14. In detail, said method comprises the steps of:

(a) contacting an agent with an isolated protein encoded by an ORF of the present invention, or nucleic acid of the invention; and

(b) determining whether the agent binds to said protein or said nucleic acid.

In general, therefore, such methods for identifying compounds that bind to a polynucleotide of the invention can comprise contacting a compound with a polynucleotide of the invention for a time sufficient to form a polynucleotide/compound complex, and detecting the complex, so that if a polynucleotide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Likewise, in general, therefore, such methods for identifying compounds that bind to a polypeptide of the invention can comprise contacting a compound with a polypeptide of the invention for a time sufficient to form a polypeptide/compound complex, and detecting the complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Methods for identifying compounds that bind to a polypeptide of the invention can also comprise contacting a compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a receptor gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds a polypeptide of the invention is identified.

Compounds identified via such methods can include compounds which modulate the activity of a polypeptide of the invention (that is, increase or decrease its activity, relative to activity observed in the absence of the compound). Alternatively, compounds identified via such methods can include compounds which modulate the expression of a polynucleotide of the invention (that is, increase or decrease expression relative to expression levels observed in the absence of the compound). Compounds, such as

compounds identified via the methods of the invention, can be tested using standard assays well known to those of skill in the art for their ability to modulate activity/expression.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W.H. Freeman, NY (1992), pp. 289-307, and Kaspczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control. One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense -

Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents. Agents which bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent, in the control of bacterial infection by modulating the activity of the protein encoded by the ORF. Agents which bind to a protein encoded by one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition.

14. USE OF NUCLEIC ACIDS AS PROBES

Another aspect of the subject invention is to provide for polypeptide-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from the nucleotide sequence of the SEQ ID NOS: 1, 12 or 14. Because the corresponding gene is only expressed in a limited number of tissues, especially adult tissues, a hybridization probe derived from SEQ ID NOS: 1, 12 or 14 can be used as an indicator of the presence of RNA of cell type of such a tissue in a sample.

Any suitable hybridization technique can be employed, such as, for example, in situ hybridization.PCR as described US Patent Nos 4,683,195 and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both. The probe will comprise a discrete nucleotide sequence for the detection of identical sequences or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means for producing specific hybridization probes for nucleic acids include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA

polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides. The nucleotide sequences may be used to construct hybridization probes for mapping their respective genomic sequences. The nucleotide sequence provided herein may be mapped to a chromosome or specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include in situ hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY.

Fluorescent in situ hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of a nucleic acid on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.The nucleotide sequence may be used to produce purified polypeptides using well known methods of recombinant DNA technology. Among the many publications that teach methods for the expression of genes after they have been isolated is Goeddel (1990) Gene Expression Technology, Methods and Enzymology, Vol 185, Academic Press, San Diego. Polypeptides may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species from which a particular polypeptide nucleotide sequence was isolated or from a different species. Advantages of producing polypeptides by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

14.1 Preparation of Sequencing Chips and Arrays

A basic example is using 6-mers attached to 50 micron surfaces to give a chip with dimensions of 3 x 3 mm which can be combined to give an array of 20 x 20 cm. Another example is using 9-mer oligonucleotides attached to 10 x 10 microns surface to

create a 9-mer chip, with dimensions of 5 x 5 mm. 4000 units of such chips may be used to create a 30 x 30 cm array. In an array in which 4,000 to 16,000 oligochips are arranged into a square array. A plate, or collection of tubes, as also depicted, may be packaged with the array as part of the sequencing kit.

The arrays may be separated physically from each other or by hydrophobic surfaces. One possible way to utilize the hydrophobic strip separation is to use technology such as the Iso-Grid Microbiology System produced by QA Laboratories, Toronto, Canada.

Hydrophobic grid membrane filters (HGMF) have been in use in analytical food microbiology for about a decade where they exhibit unique attractions of extended numerical range and automated counting of colonies. One commercially-available grid is ISO-GRID™ from QA Laboratories Ltd. (Toronto, Canada) which consists of a square (60 x 60 cm) of polysulfone polymer (Gelman Tuffryn HT-450, 0.45u pore size) on which is printed a black hydrophobic ink grid consisting of 1600 (40 x 40) square cells. HGMF have previously been inoculated with bacterial suspensions by vacuum filtration and incubated on the differential or selective media of choice.

Because the microbial growth is confined to grid cells of known position and size on the membrane, the HGMF functions more like an MPN apparatus than a conventional plate or membrane filter. Peterkin *et al.* (1987) reported that these HGMFs can be used to propagate and store genomic libraries when used with a HGMF replicator. One such instrument replicates growth from each of the 1600 cells of the ISO-GRID and enables many copies of the master HGMF to be made (Peterkin *et al.,* 1987).

Sharpe *et al.* (1989) also used ISO-GRID HGMF form QA Laboratories and an automated HGMF counter (MI-100 Interpreter) and RP-100 Replicator. They reported a technique for maintaining and screening many microbial cultures.

Peterkin and colleagues later described a method for screening DNA probes using the hydrophobic grid-membrane filter (Peterkin *et al.,* 1989). These authors reported methods for effective colony hybridization directly on HGMFs. Previously, poor results had been obtained due to the low DNA binding capacity of the epoxysulfone polymer on which the HGMFs are printed. However, Peterkin *et al.* (1989) reported that the binding of DNA to the surface of the membrane was improved by treating the replicated and incubated HGMF with polyethyleneimine, a polycation, prior to contact with DNA. Although this

early work uses cellular DNA attachment, and has a different objective to the present invention, the methodology described may be readily adapted for Format 3 SBH.

In order to identify useful sequences rapidly, Peterkin *et al.* (1989) used radiolabeled plasmid DNA from various clones and tested its specificity against the DNA on the prepared HGMFs. In this way, DNA from recombinant plasmids was rapidly screened by colony hybridization against 100 organisms on HGMF replicates which can be easily and reproducibly prepared.

Manipulation with small (2-3 mm) chips, and parallel execution of thousands of the reactions. The solution of the invention is to keep the chips and the probes in the corresponding arrays. In one example, chips containing 250,000 9-mers are synthesized on a silicon wafer in the form of 8 x 8 mM plates (15 uM/oligonucleotide, Pease et al., 1994) arrayed in 8 x 12 format (96 chips) with a 1 mM groove in between. Probes are added either by multichannel pipette or pin array, one probe on one chip. To score all 4000 6-mers, 42 chip arrays have to be used, either using different ones, or by reusing one set of chip arrays several times.

In the above case, using the earlier nomenclature of the application, F=9; P=6; and F + P = 15. Chips may have probes of formula BxNn, where x is a number of specified bases B; and n is a number of non-specified bases, so that x = 4 to 10 and n = 1 to 4. To achieve more efficient hybridization, and to avoid potential influence of any support oligonucleotides, the specified bases can be surrounded by unspecified bases, thus represented by a formula such as (N)nBx(N)m.

14.2 Preparation of Support Bound Oligonucleotides

Oligonucleotides, i.e., small nucleic acid segments, may be readily prepared by, for example, directly synthesizing the oligonucleotide by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer.

Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or Teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, 1990); using UV light (Nagata *et al.*, 1985; Dahlen *et al.*, 1987; Morriey & Collins, 1989) or by covalent

binding of base modified DNA (Keller *et al.,* 1988; 1989); all references being specifically incorporated herein.

Another strategy that may be employed is the use of the strong biotin-streptavidin interaction as a linker. For example, Broude *et al.* (1994) describe the use of Biotinylated probes, although these are duplex probes, that are immobilized on streptavidin-coated magnetic beads. Streptavidin-coated beads may be purchased from Dynal, Oslo. Of course, this same linking chemistry is applicable to coating any surface with streptavidin. Biotinylated probes may be purchased from various sources, such as, e.g., Operon Technologies (Alameda, CA).

Nunc Laboratories (Naperville, IL) is also selling suitable material that could be used. Nunc Laboratories have developed a method by which DNA can be covalently bound to the microwell surface termed Covalink NH. CovaLink NH is a polystyrene surface grafted with secondary amino groups (>NH) that serve as bridge-heads for further covalent coupling. CovaLink Modules may be purchased from Nunc Laboratories. DNA molecules may be bound to CovaLink exclusively at the 5'-end by a phosphoramidate bond, allowing immobilization of more than 1 pmol of DNA (Rasmussen *et al.,* 1991).

The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen et al., 1991). In this technology, a phosphoramidate bond is employed (Chu et al., 1983). This is beneficial as immobilization using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and then streptavidin used to bind the probes.

More specifically, the linkage method includes dissolving DNA in water (7.5 ng/ul) and denaturing for 10 min. at 95°C and cooling on ice for 10 min. Ice-cold 0.1 M 1-methylimidazole, pH 7.0 (1-$MeIm_7$), is then added to a final concentration of 10 mM 1-$MeIm_7$. A ss DNA solution is then dispensed into CovaLink NH strips (75 ul/well) standing on ice.

Carbodiimide 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), dissolved in 10 mM 1-$MeIm_7$, is made fresh and 25 ul added per well. The strips are

incubated for 5 hours at 50°C. After incubation the strips are washed using, e.g., Nunc-Immuno Wash; first the wells are washed 3 times, then they are soaked with washing solution for 5 min., and finally they are washed 3 times (where in the washing solution is 0.4 N NaOH, 0.25% SDS heated to 50°C).

It is contemplated that a further suitable method for use with the present invention is that described in PCT Patent Application WO 90/03382 (Southern & Maskos), incorporated herein by reference. This method of preparing an oligonucleotide bound to a support involves attaching a nucleoside 3'-reagent through the phosphate group by a covalent phosphodiester link to aliphatic hydroxyl groups carried by the support. The oligonucleotide is then synthesized on the supported nucleoside and protecting groups removed from the synthetic oligonucleotide chain under standard conditions that do not cleave the oligonucleotide from the support. Suitable reagents include nucleoside phosphoramidite and nucleoside hydrogen phosphorate.

An on-chip strategy for the preparation of DNA probe for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated photodeprotection may be employed in the chemical synthesis of oligonucleotides directly on a glass surface, as described by Fodor *et al.* (1991), incorporated herein by reference. Probes may also be immobilized on nylon supports as described by Van Ness *et al.* (1991); or linked to Teflon using the method of Duncan & Cavalier (1988); all references being specifically incorporated herein.

To link an oligonucleotide to a nylon support, as described by Van Ness *et al.* (1991), requires activation of the nylon surface via alkylation and selective activation of the 5'-amine of oligonucleotides with cyanuric chloride.

One particular way to prepare support bound oligonucleotides is to utilize the light-generated synthesis described by Pease *et al.,* (1994, incorporated herein by reference). These authors used current photolithographic techniques to generate arrays of immobilized oligonucleotide probes (DNA chips). These methods, in which light is used to direct the synthesis of oligonucleotide probes in high-density, miniaturized arrays, utilize photolabile 5'-protected *N*-acyl-deoxynucleoside phosphoramidites, surface linker chemistry and versatile combinatorial synthesis strategies. A matrix of 256 spatially defined oligonucleotide probes may be generated in this manner and then used in the advantageous Format 3 sequencing, as described herein.

14.3 Preparation of Nucleic Acid Fragments

The nucleic acids to be sequenced may be obtained from any appropriate source, such as cDNAs, genomic DNA, chromosomal DNA, microdissected chromosome bands, cosmid or YAC inserts, and RNA, including mRNA without any amplification steps. For example, Sambrook *et al.* (1989) describes three protocols for the isolation of high molecular weight DNA from mammalian cells (p. 9.14-9.23).

DNA fragments may be prepared as clones in M13, plasmid or lambda vectors and/or prepared directly from genomic DNA or cDNA by PCR or other amplification methods. Samples may be prepared or dispensed in multiwell plates. About 100-1000 ng of DNA samples may be prepared in 2-500 ml of final volume.

The nucleic acids would then be fragmented by any of the methods known to those of skill in the art including, for example, using restriction enzymes as described at 9.24-9.28 of Sambrook *et al.* (1989), shearing by ultrasound and NaOH treatment.

Low pressure shearing is also appropriate, as described by Schriefer *et al.* (1990, incorporated herein by reference). In this method, DNA samples are passed through a small French pressure cell at a variety of low to intermediate pressures. A lever device allows controlled application of low to intermediate pressures to the cell. The results of these studies indicate that low-pressure shearing is a useful alternative to sonic and enzymatic DNA fragmentation methods.

One particularly suitable way for fragmenting DNA is contemplated to be that using the two base recognition endonuclease, *Cvi*JI, described by Fitzgerald *et al.* (1992). These authors described an approach for the rapid fragmentation and fractionation of DNA into particular sizes that they contemplated to be suitable for shotgun cloning and sequencing. The present inventor envisions that this will also be particularly useful for generating random, but relatively small, fragments of DNA for use in the present sequencing technology.

The restriction endonuclease *Cvi*JI normally cleaves the recognition sequence PuGCPy between the G and C to leave blunt ends. Atypical reaction conditions, which alter the specificity of this enzyme (*Cvi*JI**), yield a quasi-random distribution of DNA fragments form the small molecule pUC19 (2688 base pairs). Fitzgerald *et al.* (1992) quantitatively evaluated the randomness of this fragmentation strategy, using a *Cvi*JI** digest of pUC19 that was size fractionated by a rapid gel filtration method and directly

ligated, without end repair, to a lac Z minus M13 cloning vector. Sequence analysis of 76 clones showed that *Cvi*JI** restricts pyGCPy and PuGCPu, in addition to PuGCPy sites, and that new sequence data is accumulated at a rate consistent with random fragmentation.

As reported in the literature, advantages of this approach compared to sonication and agarose gel fractionation include: smaller amounts of DNA are required (0.2-0.5 ug instead of 2-5 ug); and fewer steps are involved (no preligation, end repair, chemical extraction, or agarose gel electrophoresis and elution are needed). These advantages are also proposed to be of use when preparing DNA for sequencing by Format 3.

Irrespective of the manner in which the nucleic acid fragments are obtained or prepared, it is important to denature the DNA to give single stranded pieces available for hybridization. This is achieved by incubating the DNA solution for 2-5 minutes at 80-90°C. The solution is then cooled quickly to 2°C to prevent renaturation of the DNA fragments before they are contacted with the chip. Phosphate groups must also be removed from genomic DNA by methods known in the art.

14.4 Preparation of DNA Arrays

Arrays may be prepared by spotting DNA samples on a support such as a nylon membrane. Spotting may be performed by using arrays of metal pins (the positions of which correspond to an array of wells in a microtiter plate) to repeated by transfer of about 20 nl of a DNA solution to a nylon membrane. By offset printing, a density of dots higher than the density of the wells is achieved. One to 25 dots may be accommodated in 1 mm^2, depending on the type of label used. By avoiding spotting in some preselected number of rows and columns, separate subsets (subarrays) may be formed. Samples in one subarray may be the same genomic segment of DNA (or the same gene) from different individuals, or may be different, overlapped genomic clones. Each of the subarrays may represent replica spotting of the same samples. In one example, a selected gene segment may be amplified from 64 patients. For each patient, the amplified gene segment may be in one 96-well plate (all 96 wells containing the same sample). A plate for each of the 64 patients is prepared. By using a 96-pin device, all samples may be spotted on one 8 x 12 cm membrane. Subarrays may contain 64 samples, one from each patient. Where the 96 subarrays are identical, the dot span may be 1 mm^2 and there may be a 1 mm space between subarrays.

Another approach is to use membranes or plates (available from NUNC, Naperville, Illinois) which may be partitioned by physical spacers e.g. a plastic grid molded over the membrane, the grid being similar to the sort of membrane applied to the bottom of multiwell plates, or hydrophobic strips. A fixed physical spacer is not preferred for imaging by exposure to flat phosphor-storage screens or x-ray films.

14.5 Sequence Comparisons

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, and FASTA (Atschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990). The BLAST X program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990). The preferred computer program is FASTA version 3, specifically the FASTy program within the FASTA program package. Another preferred algorithm is the well known Smith Waterman algorithm which can also be used to determine identity.

Sequences can be compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (developed by TRW Inc., Los Angeles, CA) is used to determine regions of homology. The three parameters that determine how the sequence comparisons run are window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database can be searched for sequences containing regions of homology to the query sequence, and the appropriate sequences scored with an initial value. Subsequently, these homologous regions are examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments can be used to display the results of the homology search. Peptide and protein sequence homologies can be ascertained using the INHERIT™ 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows are used to search

protein databases for sequences containing regions of homology that were scored with an initial value. Dot-matrix homology plots can be examined to distinguish regions of significant homology from chance matches.

Alternatively, BLAST, which stands for Basic Local Alignment Search Tool, is used to search for local sequence alignments (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10). BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. Whereas it is ideal for matches which do not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP). An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search.

15. GENE THERAPY

Mutations in the polynucleotides of the invention gene may result in loss of normal function of the encoded protein. The invention thus provides gene therapy to restore normal activity of the polypeptides of the invention; or to treat disease states involving polypeptides of the invention. Delivery of a functional genes encoding polypeptides of the invention to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus), or ex vivo by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). See, for example, Anderson, Nature, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Introduction of any one of the nucleotides of the present invention or a gene

encoding the polypeptides of the present invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes. Alternatively, it is contemplated that in other human disease states, preventing the expression of or inhibiting the activity of polypeptides of the invention will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense molecules to the nucleic acids of the present invention, their complements, or their translated RNA sequences, by methods known in the art, the removal of the nucleic acids of the present invention such as using targeted deletion methods, or the insertion of a negative regulatory element such as a silencer, which is tissue specific. Further, the polypeptides of the present invention can be inhibited by the introduction of antisense molecules that hybridize to nucleic acids that encode for the polypeptides of the present invention and by the removal of a gene that encode for the polypeptides of the present invention.

The present invention still further provides cells genetically engineered *in vivo* to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell. These methods can be used to increase or decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., ada, dhfr, and the multifunctional CAD gene

which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequence include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.,* inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property

of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

16. TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention in vivo, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a polynucleotides of the invention promoter is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provid for increased protein expression. The

homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

17. THREE-DIMENSIONAL STRUCTURAL ANALYSIS

The predicted three-dimensional structure of IL-1Hy2, generated by the GeneAtlas™ program (MSI) (as described in Example 28) which includes fold predictions from Fischer and Eisenberg (*Protein Science* 5: 947-955, 1996) and homology models from Sanchez and Sali (*Proc. Natl. Acad. Sci.*, 95: 13597-13602, 1998), suggests IL-1 Hy2 is structurally related to IL-1 β and IL-1Ra. This analysis can be used to predict residues potentially involved in receptor binding and other residues important to IL-1Hy2 biological function. The three-dimensional structure of IL-1Hy2 will be useful in developing modulators of IL-1Hy2 activity such as antibodies, small molecules, peptides and derivatives thereof.

The three-dimensional structure of IL-1 Hy2 may be generated using the structural coordinates set forth below in Tables II or III. In addition, it is understood in the art that molecules or molecular complexes that are defined by the structural coordinates of Tables II or III include those plus or minus a root mean square deviation from the conserved backbone atoms of those amino acids of 2-12 Å, preferably not more than about 7 Å, or more preferably not more than about 5 Å, or most preferably not more than about 2 Å.

Identification of receptor binding residues and other residues important to IL-1 Hy2 biological function will be useful in discovering drugs which may modulate (i.e. increase or decrease) activity of the IL-1 receptor. Small molecules, antibodies and peptides which associate with one or more, or two or more, or three or more, or four or more, or five or more of the receptor binding residues or with other regions of IL-1 Hy2 may modulate IL-1Hy2 activity, *e.g.*, by increasing or decreasing its affinity for the IL-1 receptor. An understanding of the receptor binding residues and associations that occur with these residues will facilitate the development of modulators (including antagonists and agonists) of IL-1Hy2 activity, including receptor binding.

The "receptor binding residues" of IL-1 Hy2 refer to the amino acid residues of the IL-1Hy2 molecule which interact with the IL-1 receptor or any other receptor to which IL-1Hy2 binds. These amino acids preferably include Met6, Arg8, Gln17, Val27, Asp31, Val33, Pro46, Val55, Gly92, Gly93, Gln103, Ser105, and Tyr147 of SEQ ID NO: 2 and

other amino acids within 2-12 Å, preferably within 7 Å, or more preferably within 5 Å, that may interact with these listed amino acids and/or contribute to the three-dimensional conformation of these listed amino acids.

The "accessory protein binding residues" of IL-1 Hy2 refer to the amino acid residues of the IL-1 Hy2 molecule which interact with IL-1 receptor accessory protein. These amino acids preferably include Lys145 of SEQ ID NO: 2 and other amino acids within 2-12 Å, preferably within 7 Å, or more preferably within 5 Å, that may interact with this amino acid and/or contribute to the three-dimensional conformation of this amino acid.

The IL-1Hy2 three-dimensional structure allows for the generation of polypeptide variants or non-peptidyl compounds that mimic the three-dimensional structure of IL-1Hy2. The IL-1 Hy2 three-dimensional structure also allows for the identification of desirable sites for mutation to create polypeptide or non-peptidyl variants with similar, increased, decreased or different biological activity compared to wild type IL-1 Hy2. Through site-directed mutagenesis, receptor binding residues, accessory protein binding residues or other residues involved in IL-1Hy2 biological function may be mutated to create modulators of IL-1 receptor activity. The mutants may act as antagonists or agonists for the IL-1 receptor. These mutants may be useful in therapeutic compositions directed to modulating the activity of IL-1Hy2 or its receptor. These mutations can be deletions, additions or substitutions of receptor binding residues, accessory protein binding residues or other residues important to IL-1Hy2 biological function. Non-conservative substitutions are expected to be more likely to result in different biological activity compared to wild type IL-1 Hy2. For example, mutations may alter the surface charge of IL-1Hy2. The three-dimensional structure indicates that IL-1Hy2 has fewer positively charged molecules on its surface than IL-1β. Therefore, mutations of negatively charged residues on its surface to positively charged residues may alter the biological activity of IL-1Hy2. Other mutations may affect the ability of IL-1Hy2 variants (1) to bind to IL-1 receptor (IL-1R) or other receptors to which IL-1 Hy2 binds, (2) to bind to IL-1R accessory protein, or (3) ability to antagonize IL-1R. The effect of various mutations on IL-1 Hy2 activity can be modeled in three-dimensional representations on a computer using any of the computer programs described herein.

Molecular modeling may be carried out using, e.g., the structural coordinates described herein, and any computer programs known in the art. For example, programs which predict binding sites and aid in designing modulators based on three-dimensional

structural models include, but are not limited to, GRID (Oxford University) which aids in determining energetically favorable binding sites (Goodford, *J. Med. Chem.* 28: 849-857, 1985), MCSS (Molecular Simulations, Burlington, MA) which aids in determining functional maps of binding sites (Miranker and Karplus, *Proteins, Structure, Function, and Genetics*, 11: 29-34, 1991), AUTODOCK (Scripps Research) which aids in automated docking of substrates to proteins (Goodsell and Olsen, *Proteins, Structure, Function, and Genetics*, 8: 195-202, 1990, DOCK (University of San Francisco) which aids in determining macromolecular-ligand interactions (Kuntz et al., J. Mol. Biol. 161: 269-288, 1982).

The term "structure coordinates" refers to Cartesian coordinates derived from mathematical equations to generate the three-dimensional model of IL-1 Hy2 as derived from its primary amino acid sequence using, e.g., the GeneAtlas™ program. The model is used to establish the positions of the individual atoms of the IL-1 Hy2 protein.

Those of skill in the art understand that a set of structure coordinates for a molecule or a portion thereof is a relative set of points that define a structure in three dimensions. Thus, it is possible that an entirely different set of coordinates could define a similar structure. Moreover, slight variations in the individual coordinates will have little effect on overall shape. Variations in coordinates may be generated by mathematical manipulations of the structural coordinates, e.g., by permutations of the structure coordinates, fractionalization of the structure coordinates, integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any combination of the above.

Various computational analyses may be done to determine whether a molecule or a portion thereof is sufficiently similar, e.g., using current software applications, such as the Molecular Similarity application of QUANTA (Molecular Simulations Inc., San Diego, Calif.) version 4.1, and as described in the accompanying User's Guide.

The term "root mean square deviation" means the square root of the arithmetic mean of the squares of the deviations from the mean and is a way to express the deviation or variation from a trend or object. For purposes of the invention, the "root mean square deviation" defines the variation in the backbone of a protein from the polypeptide backbone of IL-1 Hy2 or a portion thereof or selected residues thereof, as substantially defined by the structural coordinates in Tables II or III below.

The present invention is illustrated in the following examples. Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader aspects of the present invention not be limited to the disclosure of the following examples. Example 1 addresses cloning of IL-1Hy2 cDNA, Example 2 addresses identification of polymorphisms, Example 3 addresses tissue expression of IL-1Hy2 mRNA and polypeptide, Example 4 addresses chromosomal localization of IL-1Hy2 DNA, Example 5 addresses identification of an IL-1 receptor binding region and binding to IL-1 receptor, Example 6 addresses IL-1Hy2 polypeptide expression in *E. coli*, Example 7 addresses confirmation of IL-1Hy2 biological activities through assessment of its modulating effect on IL-1 related activities and IL-1 related disorders, Example 8 addresses the sequencing of the IL-1Hy2 human genomic BAC clone, Example 9 addresses the sequencing of IL-1 Hy2 mouse genomic BAC clone, Example 10 addresses inhibition of IL-1β induced IL-6 production by IL-1 Hy2, Example 11 addresses the inhibition of IL-18 activity by IL-1 Hy2, Example 12 addresses IL-1 Hy2 binding to the IL-1 receptor, Example 13 addresses expression of IL-1 Hy2 in mammalian cells. Example 14 addresses the predicted three-dimensional structure of IL-1 Hy2. Example 15 addresses the crystal structure of IL-1 Hy2. Example 16 addresses site directed mutagenesis of IL-1 Hy2 based on the three-dimensional structure. Example 17 addresses expression of IL-1 Hy2 polypeptide in *E. coli*. Example 18 addresses purification of recombinant IL-Hy2 polypeptide expressed in *E. coli*.

EXAMPLE 1

Cloning of IL-1 Hy2 cDNA

A plurality of novel nucleic acids were obtained from the FSK001 cDNA library (prepared from human fetal skin tissue mRNA purchased from Invitrogen, San Diego, CA) using standard PCR, SBH sequence signature analysis and Sanger sequencing techniques. The inserts of the library were amplified with PCR using primers specific for pSport1 (GIBCO BRL, Grand Island, N.Y) vector sequences which flank the inserts. These samples were spotted onto nylon membranes and hybridized with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones were selected from each group for gel

sequencing. The 5' sequence of the amplified inserts was then deduced using the reverse M13 sequencing primer in a typical Sanger sequencing protocol. PCR products were purified and subjected to flourescent dye terminator cycle sequencing. Single pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer. One cDNA insert was identified by sequencing of several hundred base pairs (approximately 1-386 of SEQ ID NO: 1) as a novel sequence related to IL-1Ra that had not been previously reported in public databases. The remaining sequence of SEQ ID NO: 1 was obtained by further sequencing of the entire cDNA insert of the same clone; the sequence was confirmed in part by sequencing of 5' RACE PCR products from fetal skin and adult brain cDNA libraries using a Marathon cDNA amplification kit according to the manufacturer's instructions. This sequence and the clone were designated by code name CG149 and clone name RTA00003379F.h.20 (later redesignated pIL-1Hy2 and deposited at the ATCC on May 21, 1999 under Accession No. PTA-96), and the encoded polypeptide was designated IL-1Ra-Hy2 (later redesignated IL-1Hy2).

EXAMPLE 2

Identification of polymorphisms

Sequencing of a number of PCR products from various cDNA libraries revealed several potential polymorphisms, which are described with reference to the nucleotide sequence numbering of SEQ ID NO: 1.

At nucleotide 125 of SEQ ID NO: 1, the "T" may be replaced with a "C", resulting in a codon change from "GAT" to "GAC" (a silent mutation, as both codons encode the amino acid Asp). At nucleotide 184 of SEQ ID NO: 1, the "C" may be replaced with a "T", resulting in a codon change from "ACA" (encoding Thr) to "ATA" (encoding Ile). At nucleotide 205 of SEQ ID NO: 1, the "A" may be replaced with a "C", resulting in a codon change from "GAC" (Asp) to "GCC" (Ala). The changes in the amino acid sequence may be reflected in differences in the biological activities of the molecules, which can be confirmed by testing in any of the activity assays described herein.

EXAMPLE 3

Tissue Expression Study

3.1 In situ Hybridization

Gene expression of human IL-1 Hy2 was analyzed using a semi-quantitative PCR-based technique. A panel of cDNA libraries derived from human tissue (from Clontech and Invitrogen) was screened with IL-1Hy2 specific primers [5'-CCGCACCAAGGTCCCCATTTTC-3' (nucleotides 206-227) , SEQ ID NO: 10 and 3'-GAGCCCACAAGGATAACCCAGG-5' (nucleotides 728-707), SEQ ID NO: 11] to examine the mRNA expression of IL-1Hy2 in the following human tissues and cell types: heart, kidney, lung, placenta, liver, ovary, lymph node, spleen, testes, thymus, fetal liver, fetal skin, fetal spleen and macrophage. PCR assays (94 °C for 30 sec., 58 °C for 30 sec., 72 °C for 30 sec., for 30 cycles) were performed with 20 ng of cDNA derived from human tissues and cell lines and 10 picomoles of the IL-1Hy2 gene-specific primers. The 522 bp PCR product was identified through gel electrophoresis. Amplified products were separated on an agarose gel, transfered and chemically linked to a nylon filter. The filter was then hybridized with a radioactively labeled (^{33}Palpha-dCTP) double-stranded probe generated from the full-length SEQ ID NO: 1 sequence using a Klenow polymerase, random prime method. The filters were washed (high stringency) and used to expose a phosphorimaging screen for several hours. Bands indicated the presence of cDNA including SEQ ID NO: 1 sequences in a specific library, and thus mRNA expression in the corresponding cell type or tissue.

IL-1Hy2 mRNA was observed to be expressed in kidney, spleen, and fetal skin. Similar to IL-1Hy2, IL-1Ra and IL-1Hy1 mRNA are also expressed in the human fetal skin tissues, suggesting that this family of proteins may share some physiologic functions. Within the kidney, IL-1 Hy2 mRNA was detected in the distal tubules of the kidney, the glomeruli of the kidney, the Bowman's capsule epithelia, capillary epithelia, and a subset of white blood cells within the blood vessels

Additional studies were performed to localize IL-1 Hy2 mRNA expression as described by D'Andrea et al. (J. Sur. Path, 1: 191-203,1995). IL-1 Hy2 mRNA was detected in serial sections of human normal tonsil and kidney by DIG-labeled probes consisting of nucleotide 396 to 568 of SEQ ID NO: 14. The slides were hybridized with the IL-iHy2 probes for 2 hours at 54°C. Subsequently, the slides were washed with 2x SSC

at room temperature and then washed with 0.1x SSC at 54°C. After the stringency rinses, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) was used a chromagen. For visual detection, the slides were counter-stained with Eosin and examined under a light microscope.

3.2 Immunohistochemistry

The serial sections of normal tonsil were also stained with polyclonal antibodies specific for IL-1 Hy2 prepared by immunizing rabbits with IL-1 Hy2 peptide: 43-56 of SEQ. ID NO.: 2 using conventional methods [see, e.g. Harlow et al., "Antibodies: A Laboratory Manual". Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1998)] and control preimmune serum form the immunized rabbits. The resulting anti-IL-1 Hy2 antibodies did not cross react with other IL-1 family members such as IL-1Ra, Il-1β, or IL-1Hy2 on a Western Blot. In addition, the slides were also stained (via double labeling) with antibodies for CD20 (Dako, Carpenteria, CA), Ki67 (Coulter Immunotech, Miami FL), CD3, CD1a, CD14, CD68, CD45 RO and LN5. Immunohistochemistry was performed by QualTek Molecular Systems, Inc. (Santa Barbara, CA) using a modified procedure described by (Myers 1995). Antibody binding was detected with biotinylated secondary antibodies and streptavidin-AP. Fast red was used as the chromagen for detection and the slides were counter-stained with hematoxylin. IL-1 Hy2 expression was visually detected under a light microscope. For double labeling, the secondary antibodies were detected using a biotinylated secondary antibody followed by streptavidin-HRP and diaminobenzidine (DAB) was used as a chromagen. For all immunohistochemical studies a negative control was carried out in the absence of primary antibody.

In the tonsil, IL-1 Hy2 mRNA and protein were detected in a subset of B-cells (CD20 positive) in the germinal center, most of which were proliferating according to Ki67 staining suggesting that IL-1 Hy2 may play a role in regulating immune responses in the tonsil. IL-1 Hy2 was also expressed in the basal squamous epithelial of the skin surrounding the tonsil., lymph node and spleen. In a comparison of psoriatic skin and normal skin, IL-1 Hy2 polypeptide was elevated in the psoriatic skin. Furthermore, the IL-1 Hy2 positive cells did not react with anti-CD45RO (T cell marker) antibody or the anti-CD14 (monocyte marker) antibody suggesting that the IL-1 Hy2 polypeptide was not expressed in T cells or monocytes.

EXAMPLE 4

Chromosomal Localization Study

Chromosome mapping technologies allow investigators to link genes to specific regions of chromosomes. Chromosomal mapping was performed using the NIGMS human/rodent somatic cell hybrid mapping panel as described by Drwinga, H. L. et al., Genomics, 16, 311- 314, 1993 (human/rodent somatic cell hybrid mapping panel #2 purchased from the Coriell Institute for Medical Research, Camden, New Jersey). 60 ng of DNA from each sample in the panel was used as template, and 10 picomoles of the same IL-1Hy2 gene-specific oligonucleotides used in Example 3 were used as primers in a PCR assay (94 °C for 3 minutes, followed by 94 °C for 1 minute, 58 °C for 30 sec., 72 °C for 30 seconds, for 30 cycles, then 72 °C for 10 minutes). PCR products were analyzed by gel electrophoresis. The 824 bp genomic PCR product was detected only in the human/rodent somatic cell hybrid DNA containing human chromosome 2.

The IL-1 Hy2 gene was further localized using the Stanford G3 Human/Hamster Radiation panel as described by Stewart et al., *Genome Res.* 7: 422-33, (1997)(Research Genetics, Huntsville, AL). This analysis was carried out with a PCR assay as described above and localized IL-1 Hy2 gene to the 2q14 region. The gene has a 7 cRs distance from the marker SHGC-7020 and a LOD score of 10.58. The IL-1 Hy1 (marker SHGC-7020), IL-1Ra (marker W17030) IL-1β (marker SHGC-10703) were also mapped to the similar location on chromosome 2. *See* Mulero *et al., Biochem. Biophys. Res. Comm.* 263: 702-706, 1999; Smith *et al., J. Biol. Chem.,* 275: 1169-75, 2000; Kuman *et al., J. Biol. Chem.*, 275: 10308-14, 2000; Busfield *et al., Genomics* 6: 21-6, 2000; Steinlasserer *et al., Genomics* 13: 654-7, 1992; Modi *et al., Genomics* 2: 310-4, 1988; Stockman *et al., FEBS Letts.* 349: 79-83, 1994.

Gene family members are often linked to specific regions of chromosomes owing to intrachromosomal gene duplication events that give rise to multimember gene families during the process of evolution. The interleukin-1 gene family has been mapped to chromosome 2. More specifically, all of the interleukin 1 genes (IL-1α, IL-1β) and the receptors (IL-1 RI and IL-1 RII), as well as the receptor antagonist IL-1ra and the newly identified IL-1 Hy2 have been found to be situated in chromosome 2. The identification of IL-1 Hy2 sequences in this same region establishes its physical linkage to the interleukin-1 locus which indicates that IL-1 Hy2 functions as a modulator of the inflammatory response.

EXAMPLE 5

Interleukin-1 Receptor Binding Domain and Interleukin-1 Receptor Assay

The receptor binding region of both IL-1β and IL-1 Ra have been mapped to an 18 amino acid region in the carboxy terminal half of the proteins (i.e., residues 88-105 of IL-1β) by site-directed mutagenesis and protein modification studies.

IL-1 Hy2 and fragments thereof that include a receptor binding region are useful as reagents to identify cells and tissues expressing IL-1 receptors. The IL-1 receptor binding assay described in Hannum et al. Nature 343:336-340 (1990) may be used. Briefly, highly radioactive recombinant SEQ ID NOS: 2, 4 or 13 is prepared by growing *E.coli* expressing either of SEQ ID NOS: 2 ,4 or 13 on M9 medium containing [^{35}S] sulphate and purifying the labeled recombinant polypeptide by chromatography on a Mono-S column. The labeled polypeptide is incubated with the cells or tissue under standard IL-1 binding assay conditions, and [^{35}S] binding. Significant [^{35}S] binding indicates the presence of IL-1 receptors.

EXAMPLE 6

Expression of IL-1 Hy2 in E. coli

SEQ ID NOS: 1, 12 or 14 are expressed in E. coli by subcloning the entire coding region into a prokaryotic expression vector. The expression vector (pQE16) used is from the QIAexpression prokaryotic protein expression system (Qiagen). The features of this vector that make it useful for protein expression include: an efficient promoter (phage T5) to drive transcription; expression control provided by the lac operator system, which can be induced by addition of IPTG (isopropyl-β-D-thiogalactopyranoside), and an encoded His_6 tag. The latter is a stretch of 6 histidine amino acid residues which can bind very tightly to a nickel atom. The vector can be used to express a recombinant protein with a His_6 tag fused to its carboxyl terminus, allowing rapid and efficient purification using Ni-coupled affinity columns.

PCR is used to amplify the coding region which is then ligated into digested pQE16 vector. The ligation product is transformed by electroporation into electrocompetent *E.coli* cells (strain M15[pREP4] from Qiagen), and the transformed cells are plated on ampicillin-containing plates. Colonies are screened for the correct insert in the proper orientation using a PCR reaction employing a gene-specific primer and a vector-specific primer. Positives are then sequenced to ensure correct orientation and sequence. To express

IL-1 Hy2, a colony containing a correct recombinant clone is inoculated into L-Broth containing 100 µg/ml of ampicillin, 25 µg/ml of kanamycin, and the culture was allowed to grow overnight at 37°C. The saturated culture is then diluted 20-fold in the same medium and allowed to grow to an optical density at 600 nm of 0.5. At this point, IPTG is added to a final concentration of 1 mM to induce protein expression. The culture is allowed to grow for 5 more hours, and then the cells are harvested by centrifugation at 3000xg for 15 minutes.

The resultant pellet is lysed using a mild, nonionic detergent in 20mM Tris HCl (pH 7.5) (B-PER™ Reagent from Pierce), or by sonication until the turbid cell suspension turned translucent. The lysate obtained is further purified using a nickel containing column (Ni-NTA spin column from Qiagen) under non-denaturing conditions. Briefly, the lysate is brought up to 300mM NaCl and 10mM imidazole and centrifuged at 700xg through the spin column to allow the His-tagged recombinant protein to bind to the nickel column. The column is then washed twice with Wash Buffer (50mM NaH_2PO_4, pH8.0; 300mM NaCl; 20mM imidazole) and is eluted with Elution Buffer (50mM NaH_2PO_4, pH8.0; 300mM NaCl; 250mM imidazole). All the above procedures are performed at 4°C. The presence of a purified protein of the predicted size is confirmed with SDS-PAGE.

EXAMPLE 7

Evaluation of IL-1 Hy2 Activities *In Vitro and In Vivo*

7.1 Binding to the Interleukin-1 Receptor

A cell binding assay is carried out to demonstrate that IL-1 Hy2 binds to the Interleukin-1 receptor. Briefly, cell binding of the recombinant protein with and without the presence of 100-fold greater amounts of non tagged Interleukin-1 ßeta (IL-1ß) ligand is analyzed by using fluorescent antibodies specific for a IL-1 Hy2 polypeptide (e.g. specific for an express tag within the recombinant polypeptide) on the fluorescent activated cell sorter (FACS). In each reaction, 10^6 cells NHDF (normal human dermal fibroblasts) are resuspended in 100 µl of FACS buffer (distilled PBS and 3% calf serum and 0.01% azide). Cell binding is done by adding 5 nM recombinant IL-1 Hy2 in 100 µl cell suspension and as a competition in one reaction, 500 nM of recombinant IL-1 ß is also added. The cells are incubated on ice for 1 hr. The cells are pelleted, 200 µl of 0.2 mM BS3 (crosslinker) is added, and the cells are kept on ice for 30 min. Next, 10 ul 1 M Tris pH 7.5 is added and

the cells are incubated for 15 minutes on ice. The cells are pelleted, washed 1 time in FACS buffer, resuspended in 100 µl volume of FACS buffer and 2 µl primary antibody (anti-express tag antibody 1mg/ml) is added, and incubated on ice for 30 minutes. The cells are pelleted, washed with FACS buffer, and resuspended in FACS buffer (100 ul volume). The secondary antibody (phycoerythrin conjugated) 2ul of anti-mouse Ig (1mg/ml) is added and the cells are incubated for 30 minutes on ice. The cells are again pelleted, washed two times with FACS buffer, resuspended in 0.5 ml FACS buffer and analyzed on FACS. A shift in the fluorescence is expected to be observed in the cells treated with the recombinant tagged IL-1 Hy2. This binding is shown to be specific if it is competed off with the non tagged IL-1 ß protein. The results will indicate binding of IL-1 Hy2 to the IL-1 receptor.

7.2 IL-1 Antagonist Activity

IL-1 antagonist activity is determined using a prostaglandin E2 (PGE_2) based assay as follows. Cells are plated at 20,000 cells per well in a 96 well plate 24 hours before the assay. The cells are then treated with 25 pg/ml recombinant human IL-1β for 7 hours. To evaluate inhibition of IL-1β stimulated PGE_2 release by IL-1Hy2 in comparison to IL-1Ra, the cells are pretreated with various amounts of IL-1Hy2 or IL-1Ra for two hours before the addition of IL-1β. The supernatants are then collected and cell debris is removed by centrifugation. The amounts of PGE_2 in the supernatants are determined by ELISA using the PGE_2 assay system (R&D Systems) according to the manufacturer's protocol.

This assay was carried out with IL-1 Hy2 as follows. To stimulate IL-1β induced PGE_2 production, human fibroblasts (CCD 1098; accession no. CRL 2127) were plated at 20,000 cells per well in a 96 well plate 24 hours before the assay. The cells were then washed once with fresh media and incubated for 16 hours with fresh media containing 1 ng/ml recombinant human IL-1β. To evaluate inhibition of IL-1β stimulated PGE_2 release by IL-1 Hy2 in comparison to IL-1Ra, the cells were treated with various concentrations of IL-2 Hy2 or IL-1Ra together with IL-1β. After the 16 hour stimulation at 37°C in a 5%CO_2 incubator, the culture plates were centrifuged for 5 minutes at 4000 rpm to remove cellular debris. The amounts of PGE_2 were determined by assaying 100 µl of supernatant with the PGE_2 ELISA assay kit (R & D Systems) according to the manufacturer's protocol.

The addition of IL-1 Hy2 to the IL-1β stimulated cultures resulted in a dose-dependent partial decrease in PGE_2 production. At a concentration of 1000 fold excess, IL-1 Hy2 inhibited IL-1β induced PGE_2 production 40-60%. As a control and a means for

comparison, IL-1Ra completely inhibited PGE_2 production at a concentration of 100 fold excess. The fact that IL-1 Hy2 only partially inhibits IL-1β activity may be beneficial in the treatment of inflammatory disease states due to fewer side effects. It is possible that more highly purified preparations of IL-1 Hy1 may show complete inhibition in this assay.

7.3 Inhibition Of Interleukin-1 Induced Cell Proliferation

Murine D10 T cells are obtained from the American Type Culture Collection (Rockville, Md.). Cells are maintained in Dulbecco's modified Eagle medium and Ham's F-12 medium (1:1) containing 10 mM HEPES buffer (pH 7.4) and 10% fetal bovine serum. All tissue culture reagents contained less than 0.25 ng/mL endotoxin as measured by the limulus amebocyte assay.

Murine D10 cells, an Interleukin-1 dependent T-cell line, are used to measure Interleukin-1 mitogenic activity. Cell proliferation in the present of Interleukin-1 with and without the IL-1 Hy2 polypeptides of the invention is assessed by incorporation of (^{3}H) thymidine as previously described (Bakouche, O., et al. J. Immunol. 138:4249-4255, 1987). In a preferred embodiment, antagonists and agonist of the IL-1 Hy2 polypeptides of the invention are identified in this assay by adding the candidate compounds with the Interleukin-1 and IL-1 Hy2 polypeptides of the invention and measuring the change in cell proliferation caused by the candidate compound.

7.4 Inhibition Of Interleukin-1 Induced Cell Cytotoxicity

Inhibition of Interleukin-1-induced cytotoxicity is studied using an appropirate cell line, such as, for example, A375 tumor cells plated at a density of 6000 cells per well in 96-well microliter plates. After overnight attachment, Interleukin-1 (3-300 ng/mL) is added in the presence or absence of NAA or NMA. After cells are incubated for 3 days, (^{3}H) thymidine is added (1 mu Ci per well) for an additional 2 hours. Cells are harvested onto glass fiber disks (PHD Cell Harvested; Cambridge Technology, Inc., Watertown, Ma.) Disks are air dried overnight, and radioactivity is determined with a Model 1900TR Scintillation Counter (Packard Instrument Division, Downers Grove, Ill.)

7.5 Induction Of Nitrite Synthesis In Smooth Muscle Cells

Aortic smooth muscle cells are cultured by explanting segments of the medial layer of aortas from adult male Fischer 344 rats. Aortas are removed aseptically and freed of adventitial and endothelial cells by scraping both the luminal and abluminal surfaces. Medial fragments are allowed to attach to Primaria 25-cm^2 tissue culture flasks

(Becton-Dickinson, Lincoln Park, N.J.) which are kept moist with growth medium until cells emerged. Cultures are fed twice weekly with medium 199 containing 10% fetal bovine serum, 25 mM HEPES buffer (pH 7.4), 2mM L-glutamine, 40 mu g/mL endothelial cell growth supplement (Biomedical Technologies, Inc., Stoughton, Mass.) and 10 mu g/ml gentamicin (GIBCO BRL, Grand Island, N.Y.). When primary cultures become confluent, they are passaged by trypsinization, and explants are discarded. For these studies, cells from passages 12-14 are seeded at 20,000 per well in 96-well plates and are used at confluence (60,000-80,000 cells per well). The cells exhibit the classic smooth muscle cell phenotype with hill and valley morphology, and they stain positively for smooth muscle actin.

Rat aortic smooth muscle cells are incubated with RPMI-1640 medium containing 10% bovine calf serum, 25 mM HEPES buffer 7.4), 2 mM glutamine, 80 U/mL penicillin, 80 mu g/mL streptomycin, 2 mu g/mL fungizone, and Interleukin-1, IFN-gamma, and various inhibitors. At the desired times, nitrite concentration in the culture medium is measured using the standard Griess assay (Green, L., et al. Anal. Biochem. 126:131-138, 1982) adapted to a 96-well microtiter plate reader (Gross, S. S., et al. Biochem. Biophys. Res. Commun. 178:823-829, 1991). Thus, 100 muL of Griess reagent (0.5% sulfanilic acid, 0.05% naphthalenediamine, and 2.5% phosphoric acid) is added to an equal volume of culture medium, and the OD sub 550 is measured and related to nitrite concentration by reference to a standard curve. The background OD sub 550 of medium incubated in the absence of cells is subtracted from experimental values.

Rat aortic smooth muscle cells are incubated with RPMI-1640 medium containing 10% bovine calf serum, 25 mM HEPES buffer (pH 7.4), 2 mM glutamine, 80 mu g/mL penicillin, 80 mu g/mL steptomycin, 2 mu g/mL fungizone, 30 mu g/mL lipopolysaccharide (Escherichia coli 0111:B4), and 50 U/mL IFN-γ. Cells are harvested after 24 hours, and cytosol is prepared (Gross, S. S., et al. Biochem. Biophys. Res. Commun. 178:823-829, 1991). Cytosolic NO synthase activity is assayed by the Fe^{2+} -myoglobin method described previously (Gross, S. S., et al. Biochem. Biophys. Res. Commun. 178:823-829, 1991).

7.6 Alloreactivity Determined By Lymph Node Weight Gain

Experiments are conducted to show that systemic administration of the IL-1 Hy2 polypeptides of the invention suppress a localized, T cell-dependent, immune response to alloantigen presented by allogeneic cells. Mice are injected in the footpad with irradiated, allogeneic spleen cells. The mice are then injected in the contralateral footpad with

irradiated, syngeneic spleen cells. An alloreactive response (marked by proliferation of lymphocytes and inflammation) occurs in the footpad receiving the allogeneic cells, which can be measured by determining the increase in size and weight of the popliteal lymph node draining the site of antigen deposition relative to controls or by an increase in cellularity.

Specific pathogen free 8-12 week old BALB/c (H-2 sup d) and C57BL/6 (H-2 sup b) mice (Jackson Laboratory, Bar Harbor, Me.) are used in this experiment. 48 BALB/c mice are divided into 16 groups, each having 3 mice (unless otherwise indicated). Each group of mice received a different mode of treatment. On day 0 the left footpads of all mice are injected intracutaneously with 107 irradiated (2500R), allogeneic spleen cells from C57BL/6 mice in 50 ul of RPMI-1640 (Gibco) as antigen and the right contralateral footpads of the same mice are injected with 10 sup 7 irradiated (2500R), syngeneic spleen cells from BALB/c mice.

Seven days after antigen administration, the mice are sacrificed and the popliteal lymph nodes (PLN) are removed from the right and left popliteal fossa by surgical dissection. Lymph nodes are weighed and the results expressed as the difference (DELTA) in weight (mg) of the lymph node draining the site of allogeneic cell injection and the weight of the node draining the syngeneic cell injection site. Lymph nodes draining the syngeneic cell injection site weighed approximately 1 mg, regardless of whether they are obtained from mice treated with MSA or IL-1 Hy2 polypeptides of the invention, and did not differ significantly in weight from nodes obtained from mice given no cell injection.

7.7 Suppression Of Organ Graft Rejection In Vivo

Neonatal C57BL/6 (H-2 sup b) hearts are transplanted into the ear pinnae of adult BALB/c (H-2 sup d) recipients utilizing the method of Fulmer et al., Am. J. Anat. 113:273, 1963, modified as described by Trager et al., Transplantation 47:587, 1989, and Van Buren et al., Transplant. Proc. 15:2967, 1983. Survival of the transplanted hearts is assessed by visually inspecting the grafts for pulsatile activity. Pulsatile activity is determined by examining the ear-heart grafts of anesthetized recipients under a dissecting microscope with soft reflected light beginning on day 5 or 6 post transplant. The time of graft rejection is defined as the day after transplantation on which contractile activity ceases.

Recipient mice are transplanted on day 0 and injected with either IL-1 Hy2 polypeptides of the invention plus MSA (mouse serum albumin, 100 ng) or with MSA alone

on days 0 through 6, alternating i.p. and s.c. routes. In a second heart transplant experiment, the mice are injected with MSA alone on days 0 through 2, i.p. route only.

7.8 Suppression Of Inflammatory Arthritis

20 rats are divided into 4 groups, designated Groups G-J, each having 5 rats. All rats are immunized by subcutaneous injection. On day 21 following immunization with mBSA, an inflammatory arthritis response is elicited. On the same day, a negative control group is injected with a 0.2 ml volume of saline. Groups are injected with increasing amounts of IL-1 Hy2 polypeptides of the invention. Interleukin-1 is injected in one group as a positive control. The diameter of the largest egion of the treated joints is measured using a caliper on days 2, 4, 6 and 8 relative to day 0 intra-articular injection of antigen.

7.9 Activity in a Pancreatitis Model

Acute edematous, necrotizing pancreatitis is induced in adult male Swiss mice weighing more than 35 grams using caerulein--an analog of cholecystokinin. Mice are divided into four groups with three of the groups receiving caerulein 50 mu g/kg by intraperitoneal (IP) injection in four doses over three hours as previously described. (Murayama et al., Arch Surg 1990;125:1570-1572; Tani et al., International J Pancreatology 1987;2:337-348; Schoenberg et al., Free Radical Biology & Medicine 1992;12:515-522; Heath et al., Pancreas 1993;66:41-45; Saluja et al., Amer Physiological Society 1985: G702-G710; Manso et al., Digestive Disease and Sciences 1992;37:364-368). Group 1 is a control group (n-9) which receives only IP saline injections. Group 2 (n=12) is an untreated disease control. Group 3 (n=12) receives three injections of drug (10 mg/kg/hr) starting one hour prior to induction of pancreatitis. Group 4 (n=12) receives three injections of drug (10 mg/kg/hr) starting one hour after induction of pancreatitis.

After a suitable time period, all animals are euthanized, the blood collected, and the pancreata surgically excised and weighed. Serum is assayed for amylase, lipase, IL-6, and TNF levels. Each pancreas is fixed, stained, and graded histologically in a blinded fashion or interstitial edema, granulocyte infiltration, acinar vacuolization, and acinar cell. Additionally, serum levels of IL-1 Hy2 are determined, therefore allowing comparisons between dosage, serum level, systemic cytokine response, and degree of pancreatic damage.

Interleukin-6, Interleukin-1, Interleukin-1 receptor antagonist, and TNF are measured by commercially available ELISA kits (Genzyme Corp., Boston, Mass.). All

specimens are run in triplicate. Serum levels of amylase and lipase are measured on a Kodak Ectachem 700 automated analyzer (Eastman Kodak Company, Rochester, N.Y.).

Histologic slides are prepared as is known in the art after rapid excision and subsequent fixation in 10% formalin. The tissues are paraffin embedded as is known in the art and then stained with Hematoxylin and Eosin in a standard fashion. These slides are examined and graded in a blinded fashion by a board certified pathologist.

EXAMPLE 8

Sequencing of IL-1 Hy2 Human Genomic BAC Clone

To understand the genomic organization of the IL-1 Hy2 gene, a commercial human BAC library (Research Genetics) was screened by PCR with primers specifc to the full length IL-1 Hy2 cDNA using standard procedures. The BAC39316 clone containing the IL-1 Hy2 gene was partially digested with Sau3A I restriction enzyme. The resulting size-selected restriction fragments were inserted into a BamHI site of pUC18 (Pharmacia) to generate a library for screening. The BAC39316 clone containing the human genomic IL-1 Hy2 gene was sequencedusing M13 forward and reverse primers flanking the inserts. Direct BAC DNA sequencing was also carried out using primers specific to IL-1 HY2 cDNA to confirm exon/intron organization. The sequence of the BAC genomic clone is set out as SEQ ID NO: 15. Based on the sequences, exons of the IL-1 Hy2 gene were predicted using the GenScan software (Stanford University). This analysis indicated that the IL-1 Hy2 cDNA should contain additional sequences at the 5′ end in addition to those set forth in SEQ ID NO: 1.

The predicted cDNA sequence based on the genomic DNA sequence encoding IL-1 Hy2 was compared to the corresponding cDNA sequence. This analysis indicated that the predicted cDNA sequence based on the human genomic sequence of IL-1 Hy2 (SEQ ID NO: 12) contains a thymidine (T) at nucleotide 279 (see Figure 2), while the IL-1 Hy2 cDNA sequence (SEQ ID NO: 14; Figure 4) contains a cytosine (C) at position 279. The change in nucleotides (C→T) would extend the IL-1 Hy2 open reading frame in the 5′ direction, resulting in a 200 amino acid polypeptide, while the cDNA sequence (SEQ ID NO: 14; Figure 4) encodes a 152 amino acid polypeptide (SEQ ID NO: 2).

The predicted cDNA sequence (SEQ ID NO: 12) is 1366 nucleotides which contains an open reading frame (nucleotides 278 to 880) that encodes a predicted

polypeptide of 200 amino acids (SEQ ID NO: 13; Figure 2). However, the sequences surrounding the translation initiation codon at nucleotide 422 of SEQ ID NO: 12 are more similar to the Kozak translation start site consensus than the sequences surrounding nucleotide 278. Therefore, it is also possible that the IL-1 Hy2 polypeptide is encoded by a shorter open reading frame between nucleotides 422 and 880 of SEQ ID NO: 12, which encodes a predicted polypeptide of 152 amino acid (SEQ ID NO: 2).

EXAMPLE 9

Sequencing of IL-1 Hy2 Mouse Genomic BAC Clone

A commercial mouse BAC library (Research Genetics)was screened with the full length IL-1 Hy2 cDNA using standard procedures. The BAC clone containing the mouse IL-1 Hy2 gene was sequenced by conventional methods and is set forth as SEQ ID NO: 17. Based on the sequences, exons of the mouse IL-1 Hy2 gene were predicted using the GenScan software (Stanford University). This analysis indicated that the mouse IL-1Hy2 gene contains 4 exons. The predicted cDNA encoding the mouse IL-1 Hy protein is set forth as SEQ ID NO: 16. The murine IL-1 Hy2 polypeptide translation initiates at nucleotide 1 and terminates at nucleotide 457 of SEQ ID NO: 17. The mouse and human IL-1 Hy2 polypeptide sequences share 81.7% homology. The murine genomic DNA sequence can be used to generate transgenic animals which overexpress the IL-1 Hy2 polypeptide or have the IL-1 Hy2 gene knocked out as described above in Section 16.

EXAMPLE 10

Inhibition of IL-1β Induced IL-6 Production

Inhibition of Interleukin-1β induced IL-6 production was studied using human endothelial cells from umbilical vein (Huvec). Huvec cells were seeded at 2 X 10^4 cells per well in a 96-well plate the day before cell stimulation. On the day of stimulation, cells were washed once with fresh medium (F12 medium with 100 μg/ml heparin, 50 μg/ml endothelial growth supplement and 10% fetal bovine serum) and replated with 200 μl of fresh medium [without supplements] in each well. The Huvec cells were then stimulated with 100 pg/ml (final volume) of IL-1β. Although this assay was done with IL-1β, any cytokine of interest can be used. To test IL-6 inhibition, different concentrations of IL-1Hy2

(ranging from 10x to 1000x the concentration of IL-1β) or IL-1ra (ranging from 10x to 1000x IL-1β concentration) were added to the wells with the IL-1β.

After 16 hours of cell stimulation, the culture plate was spun for five minutes at 4000 rpm to remove cell debris. To test for the presence of IL-6, 100 µl of supernatant was removed and assayed with a human IL-6 immunoassay kit (R&D Systems) according to the manufacturer's instructions.

IL-1 Hy2 partially inhibited IL-1β-stimulated IL-6 production in a dose-dependent manner. In view of the fact that IL-6 blocks production of tumor necrosis factor (TNF), a pro-inflammatory cytokine, the fact that IL-1 Hy2 only partially inhibits of IL-6 production by IL-1 Hy2 may be beneficial in the treatment of inflammatory disease states with IL-1Hy2 due to reduced side effects. It is possible that more highly purified preparations of IL-1 Hy2 may show complete inhibition in this assay.

EXAMPLE 11

Inhibition of IL-18 Activity by IL-1 Hy2

The following experiment evaluated the ability of IL-1 Hy2 to inhibit IL-18 activity, as measured by induction of IFN-γ. Human lymphocytes (PBMC) were obtained by Ficoll-Hypaque density gradient separation of peripheral blood from healthy volunteer donors. Immediately after isolation, the PBMC were washed two times with growth media, containing RPMI 1640-10% fetal bovine serum, and 3 x 10^5 cells/well were seeded in a 96 well plate. The cells were stimulated by adding anti-CD3 antibody (R & D Systems, Minneapolis, MN) to all of the samples at a final concentration of 0.5 µg/ml. At the time of stimulation, all but one control well per plate were treated with 100 ng/ml recombinant IL-18 (R&D Systems) for 36 hours at 37°C at 5% CO_2. The untreated well served as a measure of background levels of IFNγ produced by stimulated PBMC cells. IL-18 treatment causes the PBMC cells to increase production of IFN-γ relative to the background levels.

To assay for IL-1 Hy2 inhibition of IL-18 stimulated IFNγ production, 100x fold to 1000x fold concentration of IL-1 Hy2 (relative to IL-18 concentration) was added to wells together with IL-18 at the time of stimulation. After 36 hours of cell stimulation, the culture plate was centrifuged for 5 minutes at 4000 rpm to remove cell debris. The supernatant was assayed for IFNγ using the Quantikine IFNγ ELISA kit (R & D Systems) according to the manufacturer's suggested protocol.

Results indicated that IL-18 alone stimulated IFNγ production and that IL-1 Hy2 had some inhibitory activities on the IL-18 stimulation. In order to assess the mechanism by which IL-1 Hy2 reduced IFNγ production, the following assay was carried out.

Human lymphocytes (PBMC) were obtained, washed, seeded, stimulated with anti-CD3 antibody and treated with a final concentration of 100 ng/ml IL-18 (R & D Systems) as described above. Several blocking antibodies were then used to test inhibition of IFNγ production, including anti-IL 18 receptor antibody, anti-IL-1 receptor accessory protein antibody, anti-IL1 receptor type I antibody and anti-IL-1 receptor type II antibody (all obtained from R & D Systems, Minneapolis, MN). Different amounts of each antibody were added to the wells with IL-18, and after 36 hours of cell stimulation, the culture plate was centrifuged for 5 minutes at 4000 rpm to remove cell debris. The supernatant was assayed for IFNγ using the Quantikine IFNγ ELISA kit (R & D Systems) according to manufacturer's instructions.

In the absence of an antibody, IL-18 stimulated IFNγ production relative to background levels as observed above. However, anti-IL18 receptor antibody, anti-accessory protein antibody and anti-IL-1 receptor type I, but not type II, antibody inhibited IL-18 induced IFNγ production.

These results indicate that compounds which antagonize the action of the IL-1 receptor inhibit IL-18 activity as measured by induction of IFNγ production.

EXAMPLE 12

Binding of IL-1 Hy2 to the Interleukin-1 Receptor

A cell binding assay was carried out, in a modification of the procedure as described above in Example 7.1, to determine if IL-1 Hy2 of the invention binds to the interleukin-1 (IL-1) receptor. Briefly, fluorescent activated cell sorting (FACS) was used to measure cell binding of the recombinant protein (see Example 6) using fluorescent antibodies specific for the express tag on the IL-1 Hy2 recombinant protein. In each reaction, 10^6 cells of human fibroblast cells (CCD 1089) were suspended in 100 μl of FACS buffer (containing distilled PBS, 3% calf serum and 0.01% azide). Cell binding reactions included 5 nM recombinant IL-1 Hy2 in 100 μl cell suspension. The cells were incubated on ice for one hour. The cells were pelleted by centrifugation, 200 μl of 0.2 mM BS3 (crosslinker) was added, and the cells were kept on ice for 30 minutes. Next, 10 μl 1 M Tris pH 7.5 was added and the cells

were incubated for 15 minutes on ice. The cells were pelleted by centrifugation, washed one time in FACS buffer, resuspended in 100 µl volume of FACS buffer, 2 µl primary antibody (anti-express tag antibody 1mg/ml) was added, and incubation continued on ice for an additional 30 minutes. The cells were pelleted by centrifugation, washed with FACS buffer, and resuspended in FACS buffer (100 µl volume). The secondary antibody (phycoerythrin-conjugated), 2 µl of anti-mouse Ig (1mg/ml), was added and the cells were incubated for 30 minutes on ice. The cells were again pelleted by centrifugation, washed two times with FACS buffer, resuspended in 0.5 ml FACS buffer and analyzed on FACS.

A shift in the fluorescence was observed for the cells treated with the recombinant tagged IL-1 Hy2. This binding was specific, as binding was not observed with the same molarity of non-related proteins, such as bovine serum albumin (BSA). Specific IL-1 Hy2 binding was also demonstrated in the murine T cell line D10 and the murine monoclonal cell line RAW 264.7. These results indicate binding of the IL-1 Hy2 protein of the invention to the IL-1 receptor.

EXAMPLE 13

Expression of IL-1 Hy2 Polypeptide in Cells

To express IL-1 Hy2 in mammalian cells, Chinese hamster ovary (CHO) cells were transfected with a mammalian expression vector and IL-1 Hy2 secretion was detected. The protein coding region of IL-1 Hy2 was obtained by PCR. The IL-1 Hy2 cDNA was used as a template for the IL-1 Hy2 specific primers (5′ GAGCCGCCATGTGTTCCCTCCCCATGGCAAG 3' and 5′ GCTACCAGC TCTGTTCAAAGT AAAAC3′; SEQ ID NO: 19 and 20 respectively) designed to amplify the shorter ORF. The PCR reaction was run for 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds. The PCR product was inserted into the pcDNA3.1/V5-His-Topo vector (Invitrogen) per manufacturer's instructions. The resulting expression construct was sequenced to confirm that the inserted IL-1 Hy2 sequence was correct.

CHO cells were obtained from the ATCC and cultured in F12K media supplemented with 10% FBS and 100 units/ml of penicillin G and 100 µg/ml streptomycin at 37°C in 5% CO_2. The CHO cells were transiently transfected with the mammalian expression vector, pcDNA IL-1 Hy2, using the FuGene transfection reagent (Roche

Molecular Biochemicals) according to manufacturer's instructions. After transfection, the medium was replaced with serum-free F12K medium The culture medium was then collected 24 hours later and passed through a 0.2 μ filter (Pall Gelman Laboratory) to remove cellular debris.

The collected conditioned medium was concentrated 10 fold using Microcon YM-10 microcolumns (Amicon) according to the manufacturer's instructions and analyzed by electrophoresis on a 15% SDS-polyacrylamide gel followed by Western blot hybridization on Immunobilon-P membrane (Millipore). IL-Hy2 was detected on the Western blot with a polyclonal antibody specific for IL-1 Hy2 using the Supersignal West Pico chemiluminescence detection reagents with goat anti-rabbit IgG conjugated with horseradish peroxidase (Pierce) as a secondary antibody. The polyclonal antibody used for the Western Blot was the IL-1 Hy2 specific antibody described in Example 3.

The IL-1 Hy2 polypeptide was detected in both the cell culture medium and in the cell lysate, suggesting that IL-1 Hy2 is a secreted polypeptide of the apparent molecular weight 25 kD when expressed in mammalian cells. The IL-1 Hy2 polypeptide expressed in mammalian cells can be sequenced to confirm the amino terminus sequence of the mature protein. Analysis of the amino acid sequence with the SignalP algorithm (Nielsen *et al., Int. J. Neural Syst.* 8:581-599, 1997) suggested that the IL-1Hy2 does not contain a signal peptide. However, some proteins such as bFGF, TGFβ, IL-1β and IL-18, are known to be secreted in the absence of a signal peptide (Nielsen *et al., supra.*) similar to IL-1Hy2.

The recombinant IL-1Hy2 protein expressed in mammalian cells (CHO has two forms, a major for of 25 kDa and a minor form of 17 kDa,, which corresponds to the predicted molecular weight (17 kDa). The increase in molecular weight may result from posttranslational modifications of the protein. The IL-1 Hy2 protein lacks N-linked glycosylation consensus sites. Neither N linked glycosylation nor O-linked glycosylation on the recombinant IL-1 Hy2 protein expressed in CHO cells was detected using peptide N-glycosidase F (PNGase F) and O-glycosidase deglycosylation analysis. Thus, the difference between the apparent molecular weight and the predicted molecular weight of IL-1 Hy2 may be due to other posttranslational modifications such as phosphorylation.

EXAMPLE 14

Three -Dimensional Structure of IL-1 Hy2

The GeneAtlas™ software package (Molecular Simulations Inc. (MSI), San Diego, CA) was used to predict the three-dimensional structure models of IL-1 Hy2. Models were generated by (1) PSI-Blast which is the multiple alignment sequence profile-based searching developed by Altschul *et al.*, (*Nucl. Acids Res.* 25: 3389-3408, 1997), (2) High Throughput Modeling (MSI) which is an automated sequence and structure searching procedure, and (3) SeqFold which is a fold recognition method described by Fischer and Eisenberg (*J. Mol. Biol.* 209: 779-791, 1998). This analysis was carried out in part by comparing the IL-1 Hy2 amino acid sequence (SEQ ID NO: 2) with the known NMR (nuclear magnetic resonance) and x-ray crystal three-dimensional structures of IL-1 Ra, IL-1 β and IL-1 α as templates. The best structural model prediction for IL-1 Hy2(highest verify score = 0.58) was based on the IL-1 β template and the results are summarized in the table I below:

Protein	Amino Acid Residues of IL-1 Hy2	Sequence Identity	Sequence Similarity	Structure Verify Score
IL-1 Ra	7-150 aa	41.1%	55.3%	0.41
IL-1 β	5-149 aa	21.2%	41.5%	**0.58**
IL-1 α	5-146 aa	19.5%	37.6%	0.38

IL-1 Hy2 was predicted to exhibit an overall β-barrel structure with a pseudo 3- fold symmetry axis down the center of the barrel. The structural models all consisted of 12 β-strands organized in three trefoil units of four antiparallel β-strands. Six of the β-strands form the barrel and the other six create a triangular array which closes the bottom of the barrel. The 12-β stranded trefoil structure is partially conserved between the IL-1 Hy2 structure models and the IL-1β, IL-1Ra and IL-1α structures . Although the IL-1 Hy2 amino acid sequence is more similar to the IL-1 Ra amino acid sequence; the IL-1 Hy2 structural model has greater structural agreement with the IL-1β structure (0.58 verify-score). The verify score produced by the MSI GeneAtlas™ program indicates the quality of the model. A verify score between 0 - 1.0, with 1 being the best, represents a good model.

The predicted three-dimensional structures of IL-1 Hy2 were superimposed with the average NMR structure of IL-1 Ra and the crystal structure of IL-1 β to evaluate

their structural differences. The structural alignment overlays of the superimposed ribbon structures are shown in Figure 5 and display similar interior core β-strands for both overlays. These overlays suggested that IL-1 Hy2 is more structurally similar to IL-1 β than IL-1 Ra. The overlay of the structural alignments also demonstrated major differences within the exterior loop region of the IL-1 Hy2 model based on IL-1 Ra.

Comparisons of the surface representations of the three-dimensional structures as viewed from surface models of the proteins suggested that IL-1 Hy2 has a similar overall shape to IL-1 β compared to the surface view of IL-1 Ra. This analysis also indicates that IL-1 Hy2 has fewer positively charged surface residues than both IL-1 β and IL-1 Ra.

Sequence alignment of IL-1 Ra and IL-1 Hy2, based on secondary structure in combination with mutagenesis analysis (*See* Boraschi *et al. Fronteriers in Bioscience* 1: 270-308, 1995), was generated as shown in Figure 6. Residues important for receptor interaction and protein function are identified in this figure with reference to the amino acid numbering of SEQ ID NO: 22 (which is missing the first 6 amino acids compared to SEQ ID NO: 2). This difference in amino acid numbering allows for the figure to correlate with the structural alignment between IL-1Ra and IL-1 Hy2.

In Figure 6, the receptor interacting amino acid are Lys7, Gln11, Asp25, Val27 and Tyr 141 and another residue identified as important for biological function is Lys139 of SEQ ID NO: 2. When the amino acid numbering of SEQ ID NO: 2 is used, the residues indicated to be associated with receptor interaction are Lys13, Gln17, Asp31, Val33 and Tyr 147 and another residue identified as important for biological function is Lys145. The corresponding Lys residue in IL-1 Ra is confirmed to be important for IL-1 Ra biological function. (*See* Boraschi *et al., supra.*). These results suggest that IL-1 Hy2 may function as an antagonist in the presence of accessory protein.

The alignment with IL-1β and IL-1 Hy2 is displayed in Figure 7. Residues important for receptor interaction and protein function are identified in this figure with reference to the amino acid numbering of SEQ ID NO: 24 (which is missing the first 4 amino acids compared to SEQ ID NO: 2). This difference in amino acid numbering allows for the figure to correlate with the structural alignment between IL-1β and IL-1 Hy2.

In Figure 7, the receptor interacting amino acid are Met2, Arg4, Lys9 Gln13, Asp27, Val27, Pro42, Val51, Gly88, Gly89, Gln99, and Ser101 and another residue

identified as important for biological function is Lys141 of SEQ ID NO: 24. When the amino acid numbering of SEQ ID NO: 2 is used, the residues indicated to be associated with receptor interaction are Met6, Arg8, Lys13, Gln17, Asp31, Val33, Pro46, Val55, Gly92, Gly93, Gln103, and Ser105 and another residue identified as important for biological function is Lys145. In this case, the importance of Lys145 also indicated that IL-1 Hy2 will function as an antagonist. Overall, this analysis suggested that IL-1 Hy2 binds to the same region of the IL-1 receptor as IL-1 β and IL-1 Ra, but IL-1 Hy2 has different atomic interactions. This suggests that IL-1 Hy2 will have a different binding affinity for the IL-1 receptor as compared to IL-1 β or IL-1 Ra.

The protein database coordinates of the IL-1 Hy2 structural models are shown in Tables II or III below. These structural coordinates were calculated based on the three-dimensional structures of IL-1 Ra and IL-1β. The protein database coordinate output file in this format provided the atom number, atom name, amino acid side chain, amino acid number, the X, Y and Z coordinates, occupancy (Occup.), the B-factor associated and 1SG with each atom. The last column indicates the row number. The "Atom Name" refers to the element whose coordinates are measured. The "Amino Acid Side Chain" refers to the name of the amino acid, and the "Amino Acid Number" refers to the position in the IL-1 Hy2 amino acid sequence in the structural model. The "X, Y and Z coordinates" refer to the atomic position measured in Angstroms. An occupancy of 1 indicates the position is fully occupied. The "B-factor" refers to the thermal factor that measures movement of the atom around the atomic center. The term "1SG" indicates the name of the structure file given by the MSI program, and the last column indicates the row number. The MSI GeneAtlas™ program used the known crystal and NMR structures of IL-1β and IL-1 Ra, respectively, as templates to derive the coordinates which can be used to generate the electron density map of IL-1 Hy2. Those of skill in the art will understand the structural coordinates set out in Figures 8 and 9 are not without standard error. The verify score produced by the MSI GeneAtlas™ program indicates the quality of the model. A verify score between 0 - 1.0, with 1 being the best, represents a good model.

Table II shows protein database coordinates for a IL-1 Hy2 structural models generated by the GeneAtlas™ Program (MSI) using the three-dimensional structure of IL-1 Ra as a template.

Table II:

	Atom			Amino Acid		X	Y	Z	Occup.	B		
		No.	Name	SC	No.					Factor		
	ATOM	1	N	ALA	1	3.198	18.691	2.923	1.00	44.80	1SG	2
	ATOM	2	CA	ALA	1	2.440	17.443	2.686	1.00	44.80	1SG	3
	ATOM	3	CB	ALA	1	2.647	16.950	1.245	1.00	44.80	1SG	4
	ATOM	4	C	ALA	1	2.894	16.372	3.614	1.00	44.80	1SG	5
	ATOM	5	O	ALA	1	3.292	16.637	4.747	1.00	44.80	1SG	6
	ATOM	6	N	ARG	2	2.837	15.112	3.148	1.00	52.08	1SG	7
	ATOM	7	CA	ARG	2	3.252	14.046	4.003	1.00	52.08	1SG	8
	ATOM	8	CB	ARG	2	2.339	12.811	3.919	1.00	52.08	1SG	9
	ATOM	9	CG	ARG	2	0.893	13.095	4.336	1.00	52.08	1SG	10
	ATOM	10	CD	ARG	2	0.592	12.800	5.807	1.00	52.08	1SG	11
	ATOM	11	NE	ARG	2	0.862	14.036	6.594	1.00	52.08	1SG	12
	ATOM	12	CZ	ARG	2	0.307	14.176	7.834	1.00	52.08	1SG	13
	ATOM	13	NH1	ARG	2	-0.491	13.188	8.334	1.00	52.08	1SG	14
	ATOM	14	NH2	ARG	2	0.552	15.299	8.570	1.00	52.08	1SG	15
	ATOM	15	C	ARG	2	4.619	13.643	3.560	1.00	52.08	1SG	16
	ATOM	16	O	ARG	2	4.805	13.131	2.458	1.00	52.08	1SG	17
	ATOM	17	N	TYR	3	5.616	13.851	4.440	1.00	57.09	1SG	18
	ATOM	18	CA	TYR	3	6.972	13.521	4.112	1.00	57.09	1SG	19
	ATOM	19	CB	TYR	3	7.982	14.591	4.558	1.00	57.09	1SG	20
	ATOM	20	CG	TYR	3	7.553	15.877	3.950	1.00	57.09	1SG	21
	ATOM	21	CD1	TYR	3	7.983	16.242	2.699	1.00	57.09	1SG	22
	ATOM	22	CD2	TYR	3	6.700	16.715	4.630	1.00	57.09	1SG	23

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor		
	ATOM	23	CE1	TYR	3	7.583	17.430	2.137	1.00	57.09	1SG	24
	ATOM	24	CE2	TYR	3	6.295	17.906	4.076	1.00	57.09	1SG	25
5	ATOM	25	CZ	TYR	3	6.738	18.264	2.826	1.00	57.09	1SG	26
	ATOM	26	OH	TYR	3	6.325	19.485	2.251	1.00	57.09	1SG	27
	ATOM	27	C	TYR	3	7.263	12.315	4.934	1.00	57.09	1SG	28
	ATOM	28	O	TYR	3	7.039	12.317	6.143	1.00	57.09	1SG	29
	ATOM	29	N	TYR	4	7.761	11.240	4.299	1.00	60.92	1SG	30
10	ATOM	30	CA	TYR	4	7.918	10.033	5.054	1.00	60.92	1SG	31
	ATOM	31	CB	TYR	4	6.891	8.982	4.592	1.00	60.92	1SG	32
	ATOM	32	CG	TYR	4	6.982	7.718	5.370	1.00	60.92	1SG	33
	ATOM	33	CD1	TYR	4	6.515	7.649	6.663	1.00	60.92	1SG	34
	ATOM	34	CD2	TYR	4	7.487	6.589	4.774	1.00	60.92	1SG	35
15	ATOM	35	CE1	TYR	4	6.589	6.470	7.365	1.00	60.92	1SG	36
	ATOM	36	CE2	TYR	4	7.560	5.414	5.469	1.00	60.92	1SG	37
	ATOM	37	CZ	TYR	4	7.118	5.353	6.764	1.00	60.92	1SG	38
	ATOM	38	OH	TYR	4	7.205	4.134	7.461	1.00	60.92	1SG	39
	ATOM	39	C	TYR	4	9.306	9.493	4.860	1.00	60.92	1SG	40
20	ATOM	40	O	TYR	4	9.853	9.552	3.759	1.00	60.92	1SG	41
	ATOM	41	N	ILE	5	9.915	8.980	5.956	1.00	62.27	1SG	42
	ATOM	42	CA	ILE	5	11.232	8.393	5.927	1.00	62.27	1SG	43
	ATOM	43	CB	ILE	5	12.286	9.357	6.414	1.00	62.27	1SG	44
	ATOM	44	CG2	ILE	5	12.123	9.477	7.937	1.00	62.27	1SG	45
25	ATOM	45	CG1	ILE	5	13.712	8.972	5.971	1.00	62.27	1SG	46
	ATOM	46	CD1	ILE	5	14.302	7.730	6.635	1.00	62.27	1SG	47
	ATOM	47	C	ILE	5	11.195	7.193	6.846	1.00	62.27	1SG	48
	ATOM	48	O	ILE	5	10.434	7.188	7.813	1.00	62.27	1SG	49
	ATOM	49	N	ILE	6	11.990	6.129	6.564	1.00	59.51	1SG	50
30	ATOM	50	CA	ILE	6	11.988	4.961	7.419	1.00	59.51	1SG	51
	ATOM	51	CB	ILE	6	11.353	3.747	6.806	1.00	59.51	1SG	52

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor		
	ATOM	52	CG2	ILE	6	9.862	4.030	6.638	1.00	59.51	1SG	53
	ATOM	53	CG1	ILE	6	12.076	3.315	5.523	1.00	59.51	1SG	54
5	ATOM	54	CD1	ILE	6	11.629	1.943	5.025	1.00	59.51	1SG	55
	ATOM	55	C	ILE	6	13.387	4.593	7.853	1.00	59.51	1SG	56
	ATOM	56	O	ILE	6	14.357	4.785	7.120	1.00	59.51	1SG	57
	ATOM	57	N	LYS	7	13.507	4.042	9.090	1.00	55.47	1SG	58
	ATOM	58	CA	LYS	7	14.777	3.738	9.700	1.00	55.47	1SG	59
10	ATOM	59	CB	LYS	7	15.293	4.999	10.421	1.00	55.47	1SG	60
	ATOM	60	CG	LYS	7	16.803	5.103	10.601	1.00	55.47	1SG	61
	ATOM	61	CD	LYS	7	17.268	6.529	10.899	1.00	55.47	1SG	62
	ATOM	62	CE	LYS	7	18.780	6.656	11.084	1.00	55.47	1SG	63
	ATOM	63	NZ	LYS	7	19.164	8.086	11.088	1.00	55.47	1SG	64
15	ATOM	64	C	LYS	7	14.560	2.624	10.705	1.00	55.47	1SG	65
	ATOM	65	O	LYS	7	13.471	2.054	10.773	1.00	55.47	1SG	66
	ATOM	66	N	TYR	8	15.612	2.232	11.471	1.00	50.67	1SG	67
	ATOM	67	CA	TYR	8	15.481	1.200	12.476	1.00	50.67	1SG	68
	ATOM	68	CB	TYR	8	16.002	-0.161	11.979	1.00	50.67	1SG	69
20	ATOM	69	CG	TYR	8	15.708	-1.222	12.985	1.00	50.67	1SG	70
	ATOM	70	CD1	TYR	8	14.449	-1.771	13.061	1.00	50.67	1SG	71
	ATOM	71	CD2	TYR	8	16.688	-1.688	13.832	1.00	50.67	1SG	72
	ATOM	72	CE1	TYR	8	14.168	-2.757	13.978	1.00	50.67	1SG	73
	ATOM	73	CE2	TYR	8	16.413	-2.674	14.751	1.00	50.67	1SG	74
25	ATOM	74	CZ	TYR	8	15.150	-3.210	14.826	1.00	50.67	1SG	75
	ATOM	75	OH	TYR	8	14.864	-4.223	15.768	1.00	50.67	1SG	76
	ATOM	76	C	TYR	8	16.308	1.606	13.670	1.00	50.67	1SG	77
	ATOM	77	O	TYR	8	17.325	2.289	13.538	1.00	50.67	1SG	78
	ATOM	78	N	ALA	9	15.888	1.147	14.869	1.00	46.42	1SG	79
30	ATOM	79	CA	ALA	9	16.457	1.494	16.146	1.00	46.42	1SG	80
	ATOM	80	CB	ALA	9	15.691	0.873	17.328	1.00	46.42	1SG	81
	ATOM	81	C	ALA	9	17.881	1.051	16.257	1.00	46.42	1SG	82

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	82 O	ALA	9	18.690	1.743	16.875	1.00	46.42	1SG 83
	ATOM	83 N	ASP	10	18.227	-0.119	15.685	1.00	45.09	1SG 84
5	ATOM	84 CA	ASP	10	19.555	-0.633	15.864	1.00	45.09	1SG 85
	ATOM	85 CB	ASP	10	19.643	-2.143	15.581	1.00	45.09	1SG 86
	ATOM	86 CG	ASP	10	18.842	-2.866	16.658	1.00	45.09	1SG 87
	ATOM	87 OD1	ASP	10	18.625	-2.265	17.743	1.00	45.09	1SG 88
	ATOM	88 OD2	ASP	10	18.431	-4.029	16.406	1.00	45.09	1SG 89
10	ATOM	89 C	ASP	10	20.505	0.053	14.933	1.00	45.09	1SG 90
	ATOM	90 O	ASP	10	21.329	-0.601	14.296	1.00	45.09	1SG 91
	ATOM	91 N	GLN	11	20.457	1.400	14.901	1.00	46.81	1SG 92
	ATOM	92 CA	GLN	11	21.339	2.232	14.128	1.00	46.81	1SG 93
	ATOM	93 CB	GLN	11	22.785	2.216	14.652	1.00	46.81	1SG 94
15	ATOM	94 CG	GLN	11	22.894	2.633	16.120	1.00	46.81	1SG 95
	ATOM	95 CD	GLN	11	22.189	3.972	16.288	1.00	46.81	1SG 96
	ATOM	96 OE1	GLN	11	22.533	4.962	15.644	1.00	46.81	1SG 97
	ATOM	97 NE2	GLN	11	21.157	3.999	17.174	1.00	46.81	1SG 98
	ATOM	98 C	GLN	11	21.375	1.838	12.679	1.00	46.81	1SG 99
20	ATOM	99 O	GLN	11	22.446	1.557	12.144	1.00	46.81	1SG 100
	ATOM	100 N	LYS	12	20.203	1.801	12.002	1.00	55.60	1SG 101
	ATOM	101 CA	LYS	12	20.173	1.494	10.596	1.00	55.60	1SG 102
	ATOM	102 CB	LYS	12	19.870	0.011	10.299	1.00	55.60	1SG 103
	ATOM	103 CG	LYS	12	20.984	-0.922	10.790	1.00	55.60	1SG 104
25	ATOM	104 CD	LYS	12	20.628	-2.413	10.764	1.00	55.60	1SG 105
	ATOM	105 CE	LYS	12	21.764	-3.313	11.262	1.00	55.60	1SG 106
	ATOM	106 NZ	LYS	12	21.446	-4.741	11.022	1.00	55.60	1SG 107
	ATOM	107 C	LYS	12	19.117	2.362	9.955	1.00	55.60	1SG 108
	ATOM	108 O	LYS	12	18.156	2.739	10.622	1.00	55.60	1SG 109
30	ATOM	109 N	ALA	13	19.275	2.709	8.646	1.00	67.26	1SG 110
	ATOM	110 CA	ALA	13	18.364	3.612	7.969	1.00	67.26	1SG 111
	ATOM	111 CB	ALA	13	18.880	5.062	7.916	1.00	67.26	1SG 112

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	112	C	ALA	13	18.144	3.192	6.531	1.00	67.26	1SG 113
ATOM	113	O	ALA	13	18.932	2.437	5.965	1.00	67.26	1SG 114
ATOM	114	N	LEU	14	17.031	3.677	5.913	1.00	79.42	1SG 115
ATOM	115	CA	LEU	14	16.674	3.399	4.538	1.00	79.42	1SG 116
ATOM	116	CB	LEU	14	15.167	3.170	4.340	1.00	79.42	1SG 117
ATOM	117	CG	LEU	14	14.779	2.791	2.901	1.00	79.42	1SG 118
ATOM	118	CD2	LEU	14	13.255	2.799	2.722	1.00	79.42	1SG 119
ATOM	119	CD1	LEU	14	15.414	1.450	2.495	1.00	79.42	1SG 120
ATOM	120	C	LEU	14	17.078	4.610	3.736	1.00	79.42	1SG 121
ATOM	121	O	LEU	14	16.950	5.739	4.213	1.00	79.42	1SG 122
ATOM	122	N	TYR	15	17.541	4.421	2.474	1.00	87.23	1SG 123
ATOM	123	CA	TYR	15	18.224	5.544	1.881	1.00	87.23	1SG 124
ATOM	124	CB	TYR	15	19.587	5.413	2.567	1.00	87.23	1SG 125
ATOM	125	CG	TYR	15	20.605	6.424	2.283	1.00	87.23	1SG 126
ATOM	126	CD1	TYR	15	21.306	6.405	1.104	1.00	87.23	1SG 127
ATOM	127	CD2	TYR	15	20.889	7.343	3.258	1.00	87.23	1SG 128
ATOM	128	CE1	TYR	15	22.274	7.345	0.870	1.00	87.23	1SG 129
ATOM	129	CE2	TYR	15	21.857	8.283	3.029	1.00	87.23	1SG 130
ATOM	130	CZ	TYR	15	22.542	8.284	1.835	1.00	87.23	1SG 131
ATOM	131	OH	TYR	15	23.538	9.251	1.599	1.00	87.23	1SG 132
ATOM	132	C	TYR	15	18.362	5.352	0.379	1.00	87.23	1SG 133
ATOM	133	O	TYR	15	18.951	4.371	-0.068	1.00	87.23	1SG 134
ATOM	134	N	THR	16	17.865	6.300	-0.452	1.00	84.17	1SG 135
ATOM	135	CA	THR	16	17.928	6.159	-1.888	1.00	84.17	1SG 136
ATOM	136	CB	THR	16	16.824	6.887	-2.595	1.00	84.17	1SG 137
ATOM	137	OG1	THR	16	16.796	6.531	-3.969	1.00	84.17	1SG 138
ATOM	138	CG2	THR	16	17.066	8.398	-2.440	1.00	84.17	1SG 139
ATOM	139	C	THR	16	19.224	6.697	-2.419	1.00	84.17	1SG 140
ATOM	140	O	THR	16	19.725	7.727	-1.976	1.00	84.17	1SG 141
ATOM	141	N	ARG	17	19.770	5.992	-3.429	1.00	78.02	1SG 142

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	142	CA	ARG	17	20.968	6.319	-4.149	1.00	78.02	1SG 143
ATOM	143	CB	ARG	17	21.962	5.148	-4.239	1.00	78.02	1SG 144
ATOM	144	CG	ARG	17	22.578	4.743	-2.897	1.00	78.02	1SG 145
ATOM	145	CD	ARG	17	23.917	5.423	-2.600	1.00	78.02	1SG 146
ATOM	146	NE	ARG	17	24.892	4.988	-3.640	1.00	78.02	1SG 147
ATOM	147	CZ	ARG	17	25.646	3.866	-3.452	1.00	78.02	1SG 148
ATOM	148	NH1	ARG	17	25.487	3.110	-2.327	1.00	78.02	1SG 149
ATOM	149	NH2	ARG	17	26.569	3.502	-4.389	1.00	78.02	1SG 150
ATOM	150	C	ARG	17	20.463	6.569	-5.531	1.00	78.02	1SG 151
ATOM	151	O	ARG	17	19.599	7.421	-5.734	1.00	78.02	1SG 152
ATOM	152	N	ASP	18	21.078	5.939	-6.548	1.00	69.34	1SG 153
ATOM	153	CA	ASP	18	20.502	6.094	-7.854	1.00	69.34	1SG 154
ATOM	154	CB	ASP	18	21.438	5.589	-8.966	1.00	69.34	1SG 155
ATOM	155	CG	ASP	18	20.761	5.794	-10.315	1.00	69.34	1SG 156
ATOM	156	OD1	ASP	18	20.559	6.976	-10.705	1.00	69.34	1SG 157
ATOM	157	OD2	ASP	18	20.435	4.771	-10.972	1.00	69.34	1SG 158
ATOM	158	C	ASP	18	19.205	5.326	-7.997	1.00	69.34	1SG 159
ATOM	159	O	ASP	18	18.126	5.913	-8.058	1.00	69.34	1SG 160
ATOM	160	N	GLY	19	19.316	3.973	-8.067	1.00	63.66	1SG 161
ATOM	161	CA	GLY	19	18.259	3.012	-8.316	1.00	63.66	1SG 162
ATOM	162	C	GLY	19	17.371	2.617	-7.167	1.00	63.66	1SG 163
ATOM	163	O	GLY	19	16.170	2.441	-7.368	1.00	63.66	1SG 164
ATOM	164	N	GLN	20	17.914	2.387	-5.950	1.00	61.51	1SG 165
ATOM	165	CA	GLN	20	17.017	1.871	-4.947	1.00	61.51	1SG 166
ATOM	166	CB	GLN	20	16.857	0.345	-4.968	1.00	61.51	1SG 167
ATOM	167	CG	GLN	20	16.150	-0.087	-6.250	1.00	61.51	1SG 168
ATOM	168	CD	GLN	20	15.734	-1.539	-6.140	1.00	61.51	1SG 169
ATOM	169	OE1	GLN	20	16.082	-2.258	-5.205	1.00	61.51	1SG 170
ATOM	170	NE2	GLN	20	14.939	-1.982	-7.150	1.00	61.51	1SG 171
ATOM	171	C	GLN	20	17.372	2.305	-3.562	1.00	61.51	1SG 172

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	172	O	GLN	20	18.374	2.981	-3.334	1.00	61.51	1SG 173
ATOM	173	N	LEU	21	16.501	1.924	-2.597	1.00	58.63	1SG 174
ATOM	174	CA	LEU	21	16.677	2.326	-1.228	1.00	58.63	1SG 175
ATOM	175	CB	LEU	21	15.347	2.472	-0.460	1.00	58.63	1SG 176
ATOM	176	CG	LEU	21	14.433	3.603	-0.981	1.00	58.63	1SG 177
ATOM	177	CD2	LEU	21	13.271	3.871	-0.015	1.00	58.63	1SG 178
ATOM	178	CD1	LEU	21	13.951	3.330	-2.416	1.00	58.63	1SG 179
ATOM	179	C	LEU	21	17.497	1.277	-0.522	1.00	58.63	1SG 180
ATOM	180	O	LEU	21	17.029	0.164	-0.288	1.00	58.63	1SG 181
ATOM	181	N	LEU	22	18.748	1.625	-0.145	1.00	56.39	1SG 182
ATOM	182	CA	LEU	22	19.654	0.716	0.512	1.00	56.39	1SG 183
ATOM	183	CB	LEU	22	21.140	1.077	0.339	1.00	56.39	1SG 184
ATOM	184	CG	LEU	22	21.627	0.859	-1.107	1.00	56.39	1SG 185
ATOM	185	CD2	LEU	22	23.157	0.944	-1.204	1.00	56.39	1SG 186
ATOM	186	CD1	LEU	22	20.901	1.793	-2.089	1.00	56.39	1SG 187
ATOM	187	C	LEU	22	19.340	0.632	1.984	1.00	56.39	1SG 188
ATOM	188	O	LEU	22	18.726	1.534	2.553	1.00	56.39	1SG 189
ATOM	189	N	VAL	23	19.776	-0.470	2.648	1.00	53.68	1SG 190
ATOM	190	CA	VAL	23	19.372	-0.692	4.014	1.00	53.68	1SG 191
ATOM	191	CB	VAL	23	18.712	-2.030	4.202	1.00	53.68	1SG 192
ATOM	192	CG1	VAL	23	18.317	-2.200	5.677	1.00	53.68	1SG 193
ATOM	193	CG2	VAL	23	17.530	-2.132	3.227	1.00	53.68	1SG 194
ATOM	194	C	VAL	23	20.541	-0.615	4.972	1.00	53.68	1SG 195
ATOM	195	O	VAL	23	21.690	-0.891	4.627	1.00	53.68	1SG 196
ATOM	196	N	GLY	24	20.240	-0.229	6.236	1.00	53.34	1SG 197
ATOM	197	CA	GLY	24	21.216	-0.178	7.293	1.00	53.34	1SG 198
ATOM	198	C	GLY	24	22.089	1.039	7.158	1.00	53.34	1SG 199
ATOM	199	O	GLY	24	21.705	2.118	7.612	1.00	53.34	1SG 200
ATOM	200	N	ASP	25	23.336	0.888	6.638	1.00	54.16	1SG 201
ATOM	201	CA	ASP	25	24.140	2.077	6.473	1.00	54.16	1SG 202

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	202	CB	ASP	25	24.891	2.522	7.738	1.00	54.16	1SG 203
	ATOM	203	CG	ASP	25	25.475	3.902	7.449	1.00	54.16	1SG 204
5	ATOM	204	OD1	ASP	25	24.942	4.584	6.533	1.00	54.16	1SG 205
	ATOM	205	OD2	ASP	25	26.452	4.296	8.141	1.00	54.16	1SG 206
	ATOM	206	C	ASP	25	25.194	1.920	5.400	1.00	54.16	1SG 207
	ATOM	207	O	ASP	25	26.380	1.894	5.724	1.00	54.16	1SG 208
	ATOM	208	N	PRO	26	24.840	1.778	4.146	1.00	53.80	1SG 209
10	ATOM	209	CA	PRO	26	25.788	1.720	3.051	1.00	53.80	1SG 210
	ATOM	210	CD	PRO	26	23.498	2.100	3.692	1.00	53.80	1SG 211
	ATOM	211	CB	PRO	26	24.929	1.637	1.790	1.00	53.80	1SG 212
	ATOM	212	CG	PRO	26	23.644	2.386	2.188	1.00	53.80	1SG 213
	ATOM	213	C	PRO	26	26.751	2.876	2.928	1.00	53.80	1SG 214
15	ATOM	214	O	PRO	26	27.922	2.713	3.260	1.00	53.80	1SG 215
	ATOM	215	N	VAL	27	26.285	4.065	2.474	1.00	51.24	1SG 216
	ATOM	216	CA	VAL	27	27.193	5.172	2.287	1.00	51.24	1SG 217
	ATOM	217	CB	VAL	27	28.095	5.012	1.095	1.00	51.24	1SG 218
	ATOM	218	CG1	VAL	27	27.230	4.971	-0.176	1.00	51.24	1SG 219
20	ATOM	219	CG2	VAL	27	29.123	6.158	1.105	1.00	51.24	1SG 220
	ATOM	220	C	VAL	27	26.410	6.431	2.058	1.00	51.24	1SG 221
	ATOM	221	O	VAL	27	25.349	6.426	1.438	1.00	51.24	1SG 222
	ATOM	222	N	ALA	28	26.947	7.565	2.549	1.00	46.54	1SG 223
	ATOM	223	CA	ALA	28	26.305	8.836	2.372	1.00	46.54	1SG 224
25	ATOM	224	CB	ALA	28	25.477	9.267	3.595	1.00	46.54	1SG 225
	ATOM	225	C	ALA	28	27.381	9.864	2.204	1.00	46.54	1SG 226
	ATOM	226	O	ALA	28	28.423	9.803	2.852	1.00	46.54	1SG 227
	ATOM	227	N	ASP	29	27.156	10.848	1.316	1.00	46.28	1SG 228
	ATOM	228	CA	ASP	29	28.119	11.890	1.127	1.00	46.28	1SG 229
30	ATOM	229	CB	ASP	29	28.440	12.177	-0.355	1.00	46.28	1SG 230
	ATOM	230	CG	ASP	29	27.174	12.563	-1.105	1.00	46.28	1SG 231
	ATOM	231	OD1	ASP	29	26.173	11.804	-1.019	1.00	46.28	1SG 232

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	232	OD2	ASP	29	27.202	13.622	-1.788	1.00	46.28	1SG 233
ATOM	233	C	ASP	29	27.575	13.114	1.788	1.00	46.28	1SG 234
ATOM	234	O	ASP	29	27.116	13.058	2.928	1.00	46.28	1SG 235
ATOM	235	N	ASN	30	27.618	14.263	1.095	1.00	48.86	1SG 236
ATOM	236	CA	ASN	30	27.164	15.467	1.716	1.00	48.86	1SG 237
ATOM	237	CB	ASN	30	27.228	16.680	0.774	1.00	48.86	1SG 238
ATOM	238	CG	ASN	30	28.692	16.993	0.510	1.00	48.86	1SG 239
ATOM	239	OD1	ASN	30	29.591	16.445	1.146	1.00	48.86	1SG 240
ATOM	240	ND2	ASN	30	28.939	17.917	-0.454	1.00	48.86	1SG 241
ATOM	241	C	ASN	30	25.735	15.292	2.117	1.00	48.86	1SG 242
ATOM	242	O	ASN	30	25.399	15.485	3.285	1.00	48.86	1SG 243
ATOM	243	N	CYS	31	24.849	14.904	1.175	1.00	56.83	1SG 244
ATOM	244	CA	CYS	31	23.482	14.739	1.582	1.00	56.83	1SG 245
ATOM	245	CB	CYS	31	22.896	15.982	2.276	1.00	56.83	1SG 246
ATOM	246	SG	CYS	31	21.167	15.751	2.786	1.00	56.83	1SG 247
ATOM	247	C	CYS	31	22.617	14.480	0.387	1.00	56.83	1SG 248
ATOM	248	O	CYS	31	22.858	15.003	-0.702	1.00	56.83	1SG 249
ATOM	249	N	CYS	32	21.582	13.639	0.592	1.00	66.99	1SG 250
ATOM	250	CA	CYS	32	20.568	13.312	-0.374	1.00	66.99	1SG 251
ATOM	251	CB	CYS	32	20.972	12.185	-1.341	1.00	66.99	1SG 252
ATOM	252	SG	CYS	32	21.327	10.625	-0.480	1.00	66.99	1SG 253
ATOM	253	C	CYS	32	19.402	12.829	0.452	1.00	66.99	1SG 254
ATOM	254	O	CYS	32	19.623	12.348	1.560	1.00	66.99	1SG 255
ATOM	255	N	ALA	33	18.141	12.888	-0.051	1.00	71.35	1SG 256
ATOM	256	CA	ALA	33	17.055	12.664	0.876	1.00	71.35	1SG 257
ATOM	257	CB	ALA	33	16.264	13.941	1.201	1.00	71.35	1SG 258
ATOM	258	C	ALA	33	16.044	11.635	0.454	1.00	71.35	1SG 259
ATOM	259	O	ALA	33	15.721	11.460	-0.720	1.00	71.35	1SG 260
ATOM	260	N	GLU	34	15.533	10.940	1.497	1.00	72.50	1SG 261
ATOM	261	CA	GLU	34	14.566	9.873	1.584	1.00	72.50	1SG 262

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM 262	CB	GLU	34	14.668	9.101	2.912	1.00	72.50	1SG 263
	ATOM 263	CG	GLU	34	13.820	7.828	2.955	1.00	72.50	1SG 264
5	ATOM 264	CD	GLU	34	14.489	6.781	2.075	1.00	72.50	1SG 265
	ATOM 265	OE1	GLU	34	15.301	7.172	1.194	1.00	72.50	1SG 266
	ATOM 266	OE2	GLU	34	14.191	5.573	2.271	1.00	72.50	1SG 267
	ATOM 267	C	GLU	34	13.137	10.333	1.435	1.00	72.50	1SG 268
	ATOM 268	O	GLU	34	12.242	9.503	1.281	1.00	72.50	1SG 269
10	ATOM 269	N	LYS	35	12.867	11.644	1.577	1.00	67.33	1SG 270
	ATOM 270	CA	LYS	35	11.538	12.209	1.559	1.00	67.33	1SG 271
	ATOM 271	CB	LYS	35	11.604	13.720	1.293	1.00	67.33	1SG 272
	ATOM 272	CG	LYS	35	10.259	14.437	1.246	1.00	67.33	1SG 273
	ATOM 273	CD	LYS	35	10.418	15.949	1.076	1.00	67.33	1SG 274
15	ATOM 274	CE	LYS	35	10.585	16.390	-0.379	1.00	67.33	1SG 275
	ATOM 275	NZ	LYS	35	10.689	17.864	-0.456	1.00	67.33	1SG 276
	ATOM 276	C	LYS	35	10.682	11.607	0.472	1.00	67.33	1SG 277
	ATOM 277	O	LYS	35	11.034	11.643	-0.705	1.00	67.33	1SG 278
	ATOM 278	N	ILE	36	9.519	11.031	0.872	1.00	61.39	1SG 279
20	ATOM 279	CA	ILE	36	8.527	10.454	-0.008	1.00	61.39	1SG 280
	ATOM 280	CB	ILE	36	8.567	8.950	-0.045	1.00	61.39	1SG 281
	ATOM 281	CG2	ILE	36	7.227	8.432	-0.591	1.00	61.39	1SG 282
	ATOM 282	CG1	ILE	36	9.765	8.467	-0.869	1.00	61.39	1SG 283
	ATOM 283	CD1	ILE	36	9.598	8.822	-2.344	1.00	61.39	1SG 284
25	ATOM 284	C	ILE	36	7.169	10.864	0.498	1.00	61.39	1SG 285
	ATOM 285	O	ILE	36	6.972	11.027	1.700	1.00	61.39	1SG 286
	ATOM 286	N	CYS	37	6.178	11.047	-0.404	1.00	56.42	1SG 287
	ATOM 287	CA	CYS	37	4.877	11.456	0.056	1.00	56.42	1SG 288
	ATOM 288	CB	CYS	37	4.174	12.446	-0.890	1.00	56.42	1SG 289
30	ATOM 289	SG	CYS	37	2.561	12.996	-0.254	1.00	56.42	1SG 290
	ATOM 290	C	CYS	37	4.009	10.237	0.179	1.00	56.42	1SG 291
	ATOM 291	O	CYS	37	3.973	9.390	-0.711	1.00	56.42	1SG 292

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	292	N	ILE	38	3.270	10.115	1.303	1.00	54.66	1SG 293
ATOM	293	CA	ILE	38	2.455	8.940	1.469	1.00	54.66	1SG 294
ATOM	294	CB	ILE	38	2.621	8.238	2.783	1.00	54.66	1SG 295
ATOM	295	CG2	ILE	38	1.543	7.142	2.853	1.00	54.66	1SG 296
ATOM	296	CG1	ILE	38	4.049	7.706	2.952	1.00	54.66	1SG 297
ATOM	297	CD1	ILE	38	4.292	7.115	4.338	1.00	54.66	1SG 298
ATOM	298	C	ILE	38	0.996	9.291	1.396	1.00	54.66	1SG 299
ATOM	299	O	ILE	38	0.532	10.192	2.093	1.00	54.66	1SG 300
ATOM	300	N	LEU	39	0.230	8.583	0.527	1.00	56.97	1SG 301
ATOM	301	CA	LEU	39	-1.197	8.788	0.479	1.00	56.97	1SG 302
ATOM	302	CB	LEU	39	-1.710	9.370	-0.848	1.00	56.97	1SG 303
ATOM	303	CG	LEU	39	-1.278	10.830	-1.083	1.00	56.97	1SG 304
ATOM	304	CD2	LEU	39	-2.066	11.476	-2.234	1.00	56.97	1SG 305
ATOM	305	CD1	LEU	39	0.244	10.944	-1.256	1.00	56.97	1SG 306
ATOM	306	C	LEU	39	-1.896	7.470	0.736	1.00	56.97	1SG 307
ATOM	307	O	LEU	39	-1.535	6.442	0.166	1.00	56.97	1SG 308
ATOM	308	N	PRO	40	-2.820	7.483	1.681	1.00	57.04	1SG 309
ATOM	309	CA	PRO	40	-3.588	6.292	2.021	1.00	57.04	1SG 310
ATOM	310	CD	PRO	40	-2.520	8.264	2.874	1.00	57.04	1SG 311
ATOM	311	CB	PRO	40	-3.585	6.209	3.547	1.00	57.04	1SG 312
ATOM	312	CG	PRO	40	-3.372	7.659	3.999	1.00	57.04	1SG 313
ATOM	313	C	PRO	40	-4.996	6.242	1.473	1.00	57.04	1SG 314
ATOM	314	O	PRO	40	-5.505	7.280	1.055	1.00	57.04	1SG 315
ATOM	315	N	ASN	41	-5.640	5.042	1.462	1.00	58.99	1SG 316
ATOM	316	CA	ASN	41	-7.033	4.905	1.092	1.00	58.99	1SG 317
ATOM	317	CB	ASN	41	-7.561	3.456	1.108	1.00	58.99	1SG 318
ATOM	318	CG	ASN	41	-8.922	3.454	0.414	1.00	58.99	1SG 319
ATOM	319	OD1	ASN	41	-9.838	4.182	0.794	1.00	58.99	1SG 320
ATOM	320	ND2	ASN	41	-9.056	2.620	-0.652	1.00	58.99	1SG 321
ATOM	321	C	ASN	41	-7.867	5.549	2.122	1.00	58.99	1SG 322

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	322	O	ASN	41	-8.703	6.411	1.852	1.00	58.99	1SG 323
	ATOM	323	N	ARG	42	-7.610	5.091	3.352	1.00	61.65	1SG 324
5	ATOM	324	CA	ARG	42	-8.332	5.431	4.528	1.00	61.65	1SG 325
	ATOM	325	CB	ARG	42	-9.650	4.661	4.658	1.00	61.65	1SG 326
	ATOM	326	CG	ARG	42	-10.645	4.972	3.540	1.00	61.65	1SG 327
	ATOM	327	CD	ARG	42	-11.899	4.102	3.586	1.00	61.65	1SG 328
	ATOM	328	NE	ARG	42	-11.461	2.693	3.386	1.00	61.65	1SG 329
10	ATOM	329	CZ	ARG	42	-12.373	1.681	3.458	1.00	61.65	1SG 330
	ATOM	330	NH1	ARG	42	-13.688	1.962	3.694	1.00	61.65	1SG 331
	ATOM	331	NH2	ARG	42	-11.966	0.387	3.295	1.00	61.65	1SG 332
	ATOM	332	C	ARG	42	-7.454	4.955	5.634	1.00	61.65	1SG 333
	ATOM	333	O	ARG	42	-6.641	5.711	6.163	1.00	61.65	1SG 334
15	ATOM	334	N	GLY	43	-7.629	3.675	6.028	1.00	65.68	1SG 335
	ATOM	335	CA	GLY	43	-6.846	3.113	7.090	1.00	65.68	1SG 336
	ATOM	336	C	GLY	43	-5.433	2.861	6.647	1.00	65.68	1SG 337
	ATOM	337	O	GLY	43	-4.947	1.733	6.713	1.00	65.68	1SG 338
	ATOM	338	N	LEU	44	-4.741	3.910	6.170	1.00	75.22	1SG 339
20	ATOM	339	CA	LEU	44	-3.331	3.886	5.885	1.00	75.22	1SG 340
	ATOM	340	CB	LEU	44	-2.491	3.268	7.018	1.00	75.22	1SG 341
	ATOM	341	CG	LEU	44	-2.541	4.040	8.347	1.00	75.22	1SG 342
	ATOM	342	CD2	LEU	44	-2.183	5.519	8.148	1.00	75.22	1SG 343
	ATOM	343	CD1	LEU	44	-1.675	3.357	9.417	1.00	75.22	1SG 344
25	ATOM	344	C	LEU	44	-3.011	3.079	4.657	1.00	75.22	1SG 345
	ATOM	345	O	LEU	44	-2.085	3.429	3.928	1.00	75.22	1SG 346
	ATOM	346	N	ASP	45	-3.809	2.027	4.368	1.00	83.45	1SG 347
	ATOM	347	CA	ASP	45	-3.608	1.109	3.276	1.00	83.45	1SG 348
	ATOM	348	CB	ASP	45	-4.325	-0.238	3.473	1.00	83.45	1SG 349
30	ATOM	349	CG	ASP	45	-3.689	-0.966	4.647	1.00	83.45	1SG 350
	ATOM	350	OD1	ASP	45	-2.519	-0.644	4.986	1.00	83.45	1SG 351
	ATOM	351	OD2	ASP	45	-4.369	-1.856	5.224	1.00	83.45	1SG 352

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	352	C	ASP	45	-4.287	1.767	2.130	1.00	83.45	1SG 353
ATOM	353	O	ASP	45	-4.119	2.971	1.985	1.00	83.45	1SG 354
ATOM	354	N	ARG	46	-4.975	0.988	1.249	1.00	85.55	1SG 355
ATOM	355	CA	ARG	46	-5.647	1.473	0.058	1.00	85.55	1SG 356
ATOM	356	CB	ARG	46	-4.986	2.742	-0.549	1.00	85.55	1SG 357
ATOM	357	CG	ARG	46	-5.950	3.634	-1.310	1.00	85.55	1SG 358
ATOM	358	CD	ARG	46	-5.648	5.146	-1.446	1.00	85.55	1SG 359
ATOM	359	NE	ARG	46	-4.193	5.443	-1.540	1.00	85.55	1SG 360
ATOM	360	CZ	ARG	46	-3.833	6.703	-1.921	1.00	85.55	1SG 361
ATOM	361	NH1	ARG	46	-4.792	7.669	-2.026	1.00	85.55	1SG 362
ATOM	362	NH2	ARG	46	-2.540	7.000	-2.226	1.00	85.55	1SG 363
ATOM	363	C	ARG	46	-5.514	0.346	-0.935	1.00	85.55	1SG 364
ATOM	364	O	ARG	46	-5.227	-0.776	-0.519	1.00	85.55	1SG 365
ATOM	365	N	THR	47	-5.712	0.588	-2.260	1.00	82.81	1SG 366
ATOM	366	CA	THR	47	-5.374	-0.404	-3.268	1.00	82.81	1SG 367
ATOM	367	CB	THR	47	-6.409	-1.482	-3.476	1.00	82.81	1SG 368
ATOM	368	OG1	THR	47	-7.687	-0.898	-3.642	1.00	82.81	1SG 369
ATOM	369	CG2	THR	47	-6.445	-2.462	-2.294	1.00	82.81	1SG 370
ATOM	370	C	THR	47	-5.069	0.262	-4.600	1.00	82.81	1SG 371
ATOM	371	O	THR	47	-5.586	1.338	-4.898	1.00	82.81	1SG 372
ATOM	372	N	LYS	48	-4.191	-0.377	-5.424	1.00	79.66	1SG 373
ATOM	373	CA	LYS	48	-3.743	0.033	-6.748	1.00	79.66	1SG 374
ATOM	374	CB	LYS	48	-4.732	0.809	-7.646	1.00	79.66	1SG 375
ATOM	375	CG	LYS	48	-4.436	0.722	-9.151	1.00	79.66	1SG 376
ATOM	376	CD	LYS	48	-5.652	0.996	-10.039	1.00	79.66	1SG 377
ATOM	377	CE	LYS	48	-6.594	-0.209	-10.142	1.00	79.66	1SG 378
ATOM	378	NZ	LYS	48	-7.750	0.112	-11.010	1.00	79.66	1SG 379
ATOM	379	C	LYS	48	-2.604	0.962	-6.582	1.00	79.66	1SG 380
ATOM	380	O	LYS	48	-1.802	0.828	-5.663	1.00	79.66	1SG 381
ATOM	381	N	VAL	49	-2.549	1.965	-7.481	1.00	77.69	1SG 382

	Atom		Amino Acid							
	No.	Name	SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	382	CA	VAL	49	-1.603	3.034	-7.410	1.00	77.69	1SG 383
	ATOM	383	CB	VAL	49	-1.910	4.144	-8.376	1.00	77.69	1SG 384
	ATOM	384	CG1	VAL	49	-0.934	5.309	-8.129	1.00	77.69	1SG 385
	ATOM	385	CG2	VAL	49	-1.860	3.567	-9.803	1.00	77.69	1SG 386
	ATOM	386	C	VAL	49	-1.745	3.536	-6.011	1.00	77.69	1SG 387
	ATOM	387	O	VAL	49	-0.772	3.947	-5.401	1.00	77.69	1SG 388
	ATOM	388	N	PRO	50	-2.928	3.481	-5.473	1.00	81.22	1SG 389
	ATOM	389	CA	PRO	50	-3.093	3.780	-4.078	1.00	81.22	1SG 390
	ATOM	390	CD	PRO	50	-4.045	4.050	-6.231	1.00	81.22	1SG 391
	ATOM	391	CB	PRO	50	-4.590	3.799	-3.931	1.00	81.22	1SG 392
	ATOM	392	CG	PRO	50	-5.073	4.492	-5.197	1.00	81.22	1SG 393
	ATOM	393	C	PRO	50	-2.280	2.994	-3.059	1.00	81.22	1SG 394
	ATOM	394	O	PRO	50	-1.577	2.067	-3.462	1.00	81.22	1SG 395
	ATOM	395	N	ILE	51	-2.440	3.350	-1.744	1.00	85.66	1SG 396
	ATOM	396	CA	ILE	51	-1.587	3.232	-0.626	1.00	85.66	1SG 397
	ATOM	397	CB	ILE	51	-1.335	1.957	0.044	1.00	85.66	1SG 398
	ATOM	398	CG2	ILE	51	-0.954	2.338	1.486	1.00	85.66	1SG 399
	ATOM	399	CG1	ILE	51	-2.496	1.010	-0.039	1.00	85.66	1SG 400
	ATOM	400	CD1	ILE	51	-2.690	0.573	-1.456	1.00	85.66	1SG 401
	ATOM	401	C	ILE	51	-0.331	3.247	-1.354	1.00	85.66	1SG 402
	ATOM	402	O	ILE	51	0.224	2.187	-1.648	1.00	85.66	1SG 403
	ATOM	403	N	PHE	52	0.153	4.443	-1.667	1.00	83.41	1SG 404
	ATOM	404	CA	PHE	52	1.377	4.400	-2.385	1.00	83.41	1SG 405
	ATOM	405	CB	PHE	52	1.338	4.682	-3.888	1.00	83.41	1SG 406
	ATOM	406	CG	PHE	52	0.997	6.115	-4.120	1.00	83.41	1SG 407
	ATOM	407	CD1	PHE	52	-0.283	6.572	-3.929	1.00	83.41	1SG 408
	ATOM	408	CD2	PHE	52	1.955	6.999	-4.558	1.00	83.41	1SG 409
	ATOM	409	CE1	PHE	52	-0.602	7.891	-4.148	1.00	83.41	1SG 410
	ATOM	410	CE2	PHE	52	1.644	8.320	-4.780	1.00	83.41	1SG 411
	ATOM	411	CZ	PHE	52	0.363	8.771	-4.572	1.00	83.41	1SG 412

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	412	C	PHE	52	2.238	5.478	-1.870	1.00	83.41	1SG 413
ATOM	413	O	PHE	52	1.887	6.191	-0.933	1.00	83.41	1SG 414
ATOM	414	N	LEU	53	3.418	5.585	-2.493	1.00	78.56	1SG 415
ATOM	415	CA	LEU	53	4.372	6.566	-2.086	1.00	78.56	1SG 416
ATOM	416	CB	LEU	53	5.612	5.924	-1.438	1.00	78.56	1SG 417
ATOM	417	CG	LEU	53	5.297	4.976	-0.261	1.00	78.56	1SG 418
ATOM	418	CD2	LEU	53	4.393	5.644	0.786	1.00	78.56	1SG 419
ATOM	419	CD1	LEU	53	6.582	4.380	0.339	1.00	78.56	1SG 420
ATOM	420	C	LEU	53	4.854	7.291	-3.317	1.00	78.56	1SG 421
ATOM	421	O	LEU	53	4.931	6.694	-4.390	1.00	78.56	1SG 422
ATOM	422	N	GLY	54	5.178	8.607	-3.220	1.00	70.60	1SG 423
ATOM	423	CA	GLY	54	5.719	9.237	-4.401	1.00	70.60	1SG 424
ATOM	424	C	GLY	54	6.195	10.638	-4.135	1.00	70.60	1SG 425
ATOM	425	O	GLY	54	5.400	11.486	-3.737	1.00	70.60	1SG 426
ATOM	426	N	ILE	55	7.517	10.892	-4.353	1.00	62.73	1SG 427
ATOM	427	CA	ILE	55	8.192	12.176	-4.273	1.00	62.73	1SG 428
ATOM	428	CB	ILE	55	8.301	12.845	-2.910	1.00	62.73	1SG 429
ATOM	429	CG2	ILE	55	9.028	14.185	-3.132	1.00	62.73	1SG 430
ATOM	430	CG1	ILE	55	6.950	13.168	-2.272	1.00	62.73	1SG 431
ATOM	431	CD1	ILE	55	6.138	14.174	-3.089	1.00	62.73	1SG 432
ATOM	432	C	ILE	55	9.616	11.941	-4.717	1.00	62.73	1SG 433
ATOM	433	O	ILE	55	9.902	11.796	-5.904	1.00	62.73	1SG 434
ATOM	434	N	GLN	56	10.545	11.904	-3.728	1.00	54.76	1SG 435
ATOM	435	CA	GLN	56	11.969	11.738	-3.875	1.00	54.76	1SG 436
ATOM	436	CB	GLN	56	12.415	10.369	-4.424	1.00	54.76	1SG 437
ATOM	437	CG	GLN	56	12.313	9.219	-3.420	1.00	54.76	1SG 438
ATOM	438	CD	GLN	56	12.904	7.975	-4.072	1.00	54.76	1SG 439
ATOM	439	OE1	GLN	56	13.861	8.062	-4.840	1.00	54.76	1SG 440
ATOM	440	NE2	GLN	56	12.319	6.788	-3.760	1.00	54.76	1SG 441
ATOM	441	C	GLN	56	12.578	12.795	-4.744	1.00	54.76	1SG 442

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	442	O	GLN	56	13.280	12.494	-5.709	1.00	54.76	1SG 443
	ATOM	443	N	GLY	57	12.282	14.073	-4.438	1.00	49.12	1SG 444
5	ATOM	444	CA	GLY	57	12.943	15.172	-5.079	1.00	49.12	1SG 445
	ATOM	445	C	GLY	57	12.076	15.715	-6.161	1.00	49.12	1SG 446
	ATOM	446	O	GLY	57	11.753	16.902	-6.167	1.00	49.12	1SG 447
	ATOM	447	N	GLY	58	11.649	14.860	-7.107	1.00	45.25	1SG 448
	ATOM	448	CA	GLY	58	10.832	15.410	-8.141	1.00	45.25	1SG 449
10	ATOM	449	C	GLY	58	10.182	14.303	-8.907	1.00	45.25	1SG 450
	ATOM	450	O	GLY	58	10.754	13.751	-9.846	1.00	45.25	1SG 451
	ATOM	451	N	SER	59	8.923	14.013	-8.531	1.00	45.65	1SG 452
	ATOM	452	CA	SER	59	8.034	13.084	-9.175	1.00	45.65	1SG 453
	ATOM	453	CB	SER	59	7.529	13.586	-10.541	1.00	45.65	1SG 454
15	ATOM	454	OG	SER	59	8.592	13.606	-11.483	1.00	45.65	1SG 455
	ATOM	455	C	SER	59	8.586	11.699	-9.376	1.00	45.65	1SG 456
	ATOM	456	O	SER	59	8.567	11.189	-10.496	1.00	45.65	1SG 457
	ATOM	457	N	ARG	60	9.088	11.039	-8.312	1.00	50.20	1SG 458
	ATOM	458	CA	ARG	60	9.455	9.657	-8.467	1.00	50.20	1SG 459
20	ATOM	459	CB	ARG	60	10.893	9.337	-8.018	1.00	50.20	1SG 460
	ATOM	460	CG	ARG	60	11.914	9.981	-8.959	1.00	50.20	1SG 461
	ATOM	461	CD	ARG	60	13.331	9.413	-8.870	1.00	50.20	1SG 462
	ATOM	462	NE	ARG	60	13.974	9.939	-7.635	1.00	50.20	1SG 463
	ATOM	463	CZ	ARG	60	15.336	9.979	-7.556	1.00	50.20	1SG 464
25	ATOM	464	NH1	ARG	60	16.091	9.573	-8.618	1.00	50.20	1SG 465
	ATOM	465	NH2	ARG	60	15.944	10.429	-6.420	1.00	50.20	1SG 466
	ATOM	466	C	ARG	60	8.466	8.869	-7.652	1.00	50.20	1SG 467
	ATOM	467	O	ARG	60	8.328	9.077	-6.449	1.00	50.20	1SG 468
	ATOM	468	N	CYS	61	7.744	7.923	-8.291	1.00	53.33	1SG 469
30	ATOM	469	CA	CYS	61	6.689	7.234	-7.595	1.00	53.33	1SG 470
	ATOM	470	CB	CYS	61	5.425	7.069	-8.464	1.00	53.33	1SG 471
	ATOM	471	SG	CYS	61	4.065	6.207	-7.620	1.00	53.33	1SG 472

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	472	C	CYS	61	7.136	5.867	-7.159	1.00	53.33	1SG 473
ATOM	473	O	CYS	61	7.627	5.070	-7.958	1.00	53.33	1SG 474
ATOM	474	N	LEU	62	6.964	5.566	-5.849	1.00	56.92	1SG 475
ATOM	475	CA	LEU	62	7.292	4.264	-5.334	1.00	56.92	1SG 476
ATOM	476	CB	LEU	62	7.707	4.220	-3.848	1.00	56.92	1SG 477
ATOM	477	CG	LEU	62	9.157	4.668	-3.573	1.00	56.92	1SG 478
ATOM	478	CD2	LEU	62	9.580	4.316	-2.138	1.00	56.92	1SG 479
ATOM	479	CD1	LEU	62	9.385	6.146	-3.916	1.00	56.92	1SG 480
ATOM	480	C	LEU	62	6.099	3.381	-5.506	1.00	56.92	1SG 481
ATOM	481	O	LEU	62	5.035	3.631	-4.937	1.00	56.92	1SG 482
ATOM	482	N	ALA	63	6.287	2.308	-6.308	1.00	55.92	1SG 483
ATOM	483	CA	ALA	63	5.249	1.375	-6.657	1.00	55.92	1SG 484
ATOM	484	CB	ALA	63	4.609	1.687	-8.021	1.00	55.92	1SG 485
ATOM	485	C	ALA	63	5.859	0.000	-6.764	1.00	55.92	1SG 486
ATOM	486	O	ALA	63	7.074	-0.166	-6.673	1.00	55.92	1SG 487
ATOM	487	N	CYS	64	5.008	-1.042	-6.909	1.00	54.10	1SG 488
ATOM	488	CA	CYS	64	5.479	-2.389	-7.086	1.00	54.10	1SG 489
ATOM	489	CB	CYS	64	4.550	-3.436	-6.452	1.00	54.10	1SG 490
ATOM	490	SG	CYS	64	4.399	-3.210	-4.655	1.00	54.10	1SG 491
ATOM	491	C	CYS	64	5.496	-2.628	-8.573	1.00	54.10	1SG 492
ATOM	492	O	CYS	64	4.482	-2.990	-9.162	1.00	54.10	1SG 493
ATOM	493	N	VAL	65	6.669	-2.465	-9.222	1.00	52.49	1SG 494
ATOM	494	CA	VAL	65	6.782	-2.513	-10.665	1.00	52.49	1SG 495
ATOM	495	CB	VAL	65	8.032	-1.844	-11.163	1.00	52.49	1SG 496
ATOM	496	CG1	VAL	65	8.114	-1.972	-12.690	1.00	52.49	1SG 497
ATOM	497	CG2	VAL	65	8.017	-0.388	-10.681	1.00	52.49	1SG 498
ATOM	498	C	VAL	65	6.777	-3.931	-11.166	1.00	52.49	1SG 499
ATOM	499	O	VAL	65	7.153	-4.855	-10.450	1.00	52.49	1SG 500
ATOM	500	N	GLU	66	6.328	-4.150	-12.427	1.00	49.66	1SG 501
ATOM	501	CA	GLU	66	6.253	-5.496	-12.918	1.00	49.66	1SG 502

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	502	CB	GLU	66	4.897	-5.874	-13.549	1.00	49.66	1SG 503
ATOM	503	CG	GLU	66	3.727	-5.980	-12.564	1.00	49.66	1SG 504
ATOM	504	CD	GLU	66	3.125	-4.595	-12.354	1.00	49.66	1SG 505
ATOM	505	OE1	GLU	66	3.826	-3.708	-11.796	1.00	49.66	1SG 506
ATOM	506	OE2	GLU	66	1.946	-4.407	-12.755	1.00	49.66	1SG 507
ATOM	507	C	GLU	66	7.281	-5.726	-13.967	1.00	49.66	1SG 508
ATOM	508	O	GLU	66	7.236	-5.144	-15.051	1.00	49.66	1SG 509
ATOM	509	N	THR	67	8.259	-6.592	-13.652	1.00	47.84	1SG 510
ATOM	510	CA	THR	67	9.128	-7.002	-14.700	1.00	47.84	1SG 511
ATOM	511	CB	THR	67	10.473	-7.508	-14.265	1.00	47.84	1SG 512
ATOM	512	OG1	THR	67	11.353	-7.552	-15.378	1.00	47.84	1SG 513
ATOM	513	CG2	THR	67	10.310	-8.924	-13.694	1.00	47.84	1SG 514
ATOM	514	C	THR	67	8.355	-8.150	-15.239	1.00	47.84	1SG 515
ATOM	515	O	THR	67	7.242	-8.397	-14.779	1.00	47.84	1SG 516
ATOM	516	N	GLU	68	8.852	-8.870	-16.250	1.00	46.72	1SG 517
ATOM	517	CA	GLU	68	8.000	-9.946	-16.660	1.00	46.72	1SG 518
ATOM	518	CB	GLU	68	8.509	-10.736	-17.877	1.00	46.72	1SG 519
ATOM	519	CG	GLU	68	7.553	-11.873	-18.259	1.00	46.72	1SG 520
ATOM	520	CD	GLU	68	8.228	-12.752	-19.301	1.00	46.72	1SG 521
ATOM	521	OE1	GLU	68	9.049	-12.210	-20.088	1.00	46.72	1SG 522
ATOM	522	OE2	GLU	68	7.938	-13.979	-19.319	1.00	46.72	1SG 523
ATOM	523	C	GLU	68	7.902	-10.940	-15.548	1.00	46.72	1SG 524
ATOM	524	O	GLU	68	6.805	-11.305	-15.125	1.00	46.72	1SG 525
ATOM	525	N	GLU	69	9.067	-11.369	-15.027	1.00	46.10	1SG 526
ATOM	526	CA	GLU	69	9.118	-12.419	-14.051	1.00	46.10	1SG 527
ATOM	527	CB	GLU	69	10.565	-12.836	-13.758	1.00	46.10	1SG 528
ATOM	528	CG	GLU	69	11.352	-13.198	-15.019	1.00	46.10	1SG 529
ATOM	529	CD	GLU	69	11.792	-11.888	-15.664	1.00	46.10	1SG 530
ATOM	530	OE1	GLU	69	12.402	-11.055	-14.939	1.00	46.10	1SG 531
ATOM	531	OE2	GLU	69	11.527	-11.698	-16.880	1.00	46.10	1SG 532

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	532	C	GLU	69	8.528	-12.003	-12.742	1.00	46.10	1SG 533
	ATOM	533	O	GLU	69	7.571	-12.599	-12.251	1.00	46.10	1SG 534
5	ATOM	534	N	GLY	70	9.034	-10.915	-12.146	1.00	46.58	1SG 535
	ATOM	535	CA	GLY	70	8.525	-10.675	-10.832	1.00	46.58	1SG 536
	ATOM	536	C	GLY	70	8.540	-9.209	-10.542	1.00	46.58	1SG 537
	ATOM	537	O	GLY	70	9.152	-8.419	-11.258	1.00	46.58	1SG 538
	ATOM	538	N	PRO	71	7.776	-8.841	-9.541	1.00	48.89	1SG 539
10	ATOM	539	CA	PRO	71	7.766	-7.452	-9.141	1.00	48.89	1SG 540
	ATOM	540	CD	PRO	71	6.437	-9.409	-9.481	1.00	48.89	1SG 541
	ATOM	541	CB	PRO	71	6.301	-7.073	-8.920	1.00	48.89	1SG 542
	ATOM	542	CG	PRO	71	5.599	-8.411	-8.670	1.00	48.89	1SG 543
	ATOM	543	C	PRO	71	8.623	-7.095	-7.949	1.00	48.89	1SG 544
15	ATOM	544	O	PRO	71	8.676	-7.874	-6.997	1.00	48.89	1SG 545
	ATOM	545	N	SER	72	9.202	-5.869	-7.937	1.00	52.25	1SG 546
	ATOM	546	CA	SER	72	10.027	-5.386	-6.858	1.00	52.25	1SG 547
	ATOM	547	CB	SER	72	11.533	-5.496	-7.156	1.00	52.25	1SG 548
	ATOM	548	OG	SER	72	11.900	-6.860	-7.305	1.00	52.25	1SG 549
20	ATOM	549	C	SER	72	9.716	-3.925	-6.660	1.00	52.25	1SG 550
	ATOM	550	O	SER	72	9.029	-3.313	-7.477	1.00	52.25	1SG 551
	ATOM	551	N	LEU	73	10.200	-3.332	-5.543	1.00	56.41	1SG 552
	ATOM	552	CA	LEU	73	9.942	-1.944	-5.243	1.00	56.41	1SG 553
	ATOM	553	CB	LEU	73	10.215	-1.624	-3.763	1.00	56.41	1SG 554
25	ATOM	554	CG	LEU	73	9.954	-0.167	-3.358	1.00	56.41	1SG 555
	ATOM	555	CD2	LEU	73	10.384	0.077	-1.905	1.00	56.41	1SG 556
	ATOM	556	CD1	LEU	73	8.493	0.230	-3.610	1.00	56.41	1SG 557
	ATOM	557	C	LEU	73	10.809	-1.069	-6.107	1.00	56.41	1SG 558
	ATOM	558	O	LEU	73	12.017	-1.286	-6.209	1.00	56.41	1SG 559
30	ATOM	559	N	GLN	74	10.210	-0.035	-6.745	1.00	58.13	1SG 560
	ATOM	560	CA	GLN	74	10.980	0.815	-7.611	1.00	58.13	1SG 561
	ATOM	561	CB	GLN	74	10.964	0.374	-9.077	1.00	58.13	1SG 562

	Atom No.	Name	Amino Acid SC	No.		X	Y	Z	Occup.	B Factor	
	ATOM	562	CG	GLN	74	11.793	1.311	-9.948	1.00	58.13	1SG 563
	ATOM	563	CD	GLN	74	11.570	0.904	-11.386	1.00	58.13	1SG 564
5	ATOM	564	OE1	GLN	74	11.127	-0.211	-11.655	1.00	58.13	1SG 565
	ATOM	565	NE2	GLN	74	11.874	1.835	-12.329	1.00	58.13	1SG 566
	ATOM	566	C	GLN	74	10.436	2.215	-7.604	1.00	58.13	1SG 567
	ATOM	567	O	GLN	74	9.254	2.440	-7.341	1.00	58.13	1SG 568
	ATOM	568	N	LEU	75	11.317	3.201	-7.899	1.00	57.60	1SG 569
10	ATOM	569	CA	LEU	75	10.922	4.581	-8.000	1.00	57.60	1SG 570
	ATOM	570	CB	LEU	75	11.865	5.553	-7.251	1.00	57.60	1SG 571
	ATOM	571	CG	LEU	75	13.345	5.118	-7.120	1.00	57.60	1SG 572
	ATOM	572	CD2	LEU	75	14.044	4.945	-8.474	1.00	57.60	1SG 573
	ATOM	573	CD1	LEU	75	13.486	3.883	-6.217	1.00	57.60	1SG 574
15	ATOM	574	C	LEU	75	10.861	4.943	-9.459	1.00	57.60	1SG 575
	ATOM	575	O	LEU	75	11.863	5.291	-10.081	1.00	57.60	1SG 576
	ATOM	576	N	GLU	76	9.646	4.903	-10.042	1.00	53.82	1SG 577
	ATOM	577	CA	GLU	76	9.479	5.166	-11.445	1.00	53.82	1SG 578
	ATOM	578	CB	GLU	76	8.308	4.373	-12.064	1.00	53.82	1SG 579
20	ATOM	579	CG	GLU	76	6.959	4.659	-11.394	1.00	53.82	1SG 580
	ATOM	580	CD	GLU	76	5.880	3.832	-12.083	1.00	53.82	1SG 581
	ATOM	581	OE1	GLU	76	5.747	2.627	-11.732	1.00	53.82	1SG 582
	ATOM	582	OE2	GLU	76	5.180	4.388	-12.969	1.00	53.82	1SG 583
	ATOM	583	C	GLU	76	9.207	6.631	-11.635	1.00	53.82	1SG 584
25	ATOM	584	O	GLU	76	8.479	7.245	-10.857	1.00	53.82	1SG 585
	ATOM	585	N	ASP	77	9.805	7.235	-12.687	1.00	49.18	1SG 586
	ATOM	586	CA	ASP	77	9.612	8.637	-12.940	1.00	49.18	1SG 587
	ATOM	587	CB	ASP	77	10.685	9.258	-13.853	1.00	49.18	1SG 588
	ATOM	588	CG	ASP	77	11.993	9.332	-13.077	1.00	49.18	1SG 589
30	ATOM	589	OD1	ASP	77	12.007	8.894	-11.896	1.00	49.18	1SG 590
	ATOM	590	OD2	ASP	77	12.994	9.835	-13.654	1.00	49.18	1SG 591
	ATOM	591	C	ASP	77	8.293	8.804	-13.624	1.00	49.18	1SG 592

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	592	O	ASP	77	8.091	8.307	-14.731	1.00	49.18	1SG 593
	ATOM	593	N	VAL	78	7.353	9.521	-12.973	1.00	47.59	1SG 594
5	ATOM	594	CA	VAL	78	6.057	9.685	-13.568	1.00	47.59	1SG 595
	ATOM	595	CB	VAL	78	4.923	9.505	-12.596	1.00	47.59	1SG 596
	ATOM	596	CG1	VAL	78	5.001	10.596	-11.514	1.00	47.59	1SG 597
	ATOM	597	CG2	VAL	78	3.601	9.479	-13.384	1.00	47.59	1SG 598
	ATOM	598	C	VAL	78	5.963	11.043	-14.201	1.00	47.59	1SG 599
10	ATOM	599	O	VAL	78	6.092	12.078	-13.548	1.00	47.59	1SG 600
	ATOM	600	N	ASN	79	5.734	11.042	-15.528	1.00	47.00	1SG 601
	ATOM	601	CA	ASN	79	5.658	12.196	-16.382	1.00	47.00	1SG 602
	ATOM	602	CB	ASN	79	5.700	11.835	-17.874	1.00	47.00	1SG 603
	ATOM	603	CG	ASN	79	7.120	11.417	-18.226	1.00	47.00	1SG 604
15	ATOM	604	OD1	ASN	79	7.421	10.232	-18.354	1.00	47.00	1SG 605
	ATOM	605	ND2	ASN	79	8.020	12.421	-18.400	1.00	47.00	1SG 606
	ATOM	606	C	ASN	79	4.419	13.016	-16.161	1.00	47.00	1SG 607
	ATOM	607	O	ASN	79	4.432	14.219	-16.420	1.00	47.00	1SG 608
	ATOM	608	N	ILE	80	3.305	12.405	-15.708	1.00	48.12	1SG 609
20	ATOM	609	CA	ILE	80	2.066	13.141	-15.658	1.00	48.12	1SG 610
	ATOM	610	CB	ILE	80	0.899	12.322	-16.127	1.00	48.12	1SG 611
	ATOM	611	CG2	ILE	80	0.788	11.081	-15.226	1.00	48.12	1SG 612
	ATOM	612	CG1	ILE	80	-0.373	13.179	-16.180	1.00	48.12	1SG 613
	ATOM	613	CD1	ILE	80	-1.524	12.502	-16.917	1.00	48.12	1SG 614
25	ATOM	614	C	ILE	80	1.747	13.618	-14.268	1.00	48.12	1SG 615
	ATOM	615	O	ILE	80	1.691	12.840	-13.319	1.00	48.12	1SG 616
	ATOM	616	N	GLU	81	1.578	14.950	-14.125	1.00	50.04	1SG 617
	ATOM	617	CA	GLU	81	1.244	15.589	-12.880	1.00	50.04	1SG 618
	ATOM	618	CB	GLU	81	1.532	17.100	-12.916	1.00	50.04	1SG 619
30	ATOM	619	CG	GLU	81	3.004	17.437	-13.183	1.00	50.04	1SG 620
	ATOM	620	CD	GLU	81	3.861	16.850	-12.068	1.00	50.04	1SG 621
	ATOM	621	OE1	GLU	81	3.873	15.598	-11.925	1.00	50.04	1SG 622

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	622	OE2	GLU	81	4.523	17.646	-11.350	1.00	50.04	1SG 623
	ATOM	623	C	GLU	81	-0.206	15.397	-12.512	1.00	50.04	1SG 624
5	ATOM	624	O	GLU	81	-0.535	15.164	-11.350	1.00	50.04	1SG 625
	ATOM	625	N	GLU	82	-1.114	15.502	-13.504	1.00	52.62	1SG 626
	ATOM	626	CA	GLU	82	-2.533	15.506	-13.258	1.00	52.62	1SG 627
	ATOM	627	CB	GLU	82	-3.346	15.831	-14.525	1.00	52.62	1SG 628
	ATOM	628	CG	GLU	82	-4.840	16.034	-14.263	1.00	52.62	1SG 629
10	ATOM	629	CD	GLU	82	-5.532	14.679	-14.304	1.00	52.62	1SG 630
	ATOM	630	OE1	GLU	82	-5.494	14.034	-15.386	1.00	52.62	1SG 631
	ATOM	631	OE2	GLU	82	-6.107	14.270	-13.260	1.00	52.62	1SG 632
	ATOM	632	C	GLU	82	-3.027	14.200	-12.721	1.00	52.62	1SG 633
	ATOM	633	O	GLU	82	-3.775	14.175	-11.744	1.00	52.62	1SG 634
15	ATOM	634	N	LEU	83	-2.610	13.075	-13.326	1.00	56.83	1SG 635
	ATOM	635	CA	LEU	83	-3.112	11.795	-12.915	1.00	56.83	1SG 636
	ATOM	636	CB	LEU	83	-2.651	10.687	-13.886	1.00	56.83	1SG 637
	ATOM	637	CG	LEU	83	-3.003	9.238	-13.503	1.00	56.83	1SG 638
	ATOM	638	CD2	LEU	83	-4.505	9.074	-13.230	1.00	56.83	1SG 639
20	ATOM	639	CD1	LEU	83	-2.122	8.732	-12.351	1.00	56.83	1SG 640
	ATOM	640	C	LEU	83	-2.614	11.512	-11.534	1.00	56.83	1SG 641
	ATOM	641	O	LEU	83	-1.410	11.435	-11.296	1.00	56.83	1SG 642
	ATOM	642	N	TYR	84	-3.549	11.364	-10.573	1.00	63.40	1SG 643
	ATOM	643	CA	TYR	84	-3.154	11.085	-9.224	1.00	63.40	1SG 644
25	ATOM	644	CB	TYR	84	-3.212	12.343	-8.343	1.00	63.40	1SG 645
	ATOM	645	CG	TYR	84	-2.238	12.180	-7.233	1.00	63.40	1SG 646
	ATOM	646	CD1	TYR	84	-2.491	11.399	-6.130	1.00	63.40	1SG 647
	ATOM	647	CD2	TYR	84	-1.036	12.843	-7.325	1.00	63.40	1SG 648
	ATOM	648	CE1	TYR	84	-1.544	11.291	-5.135	1.00	63.40	1SG 649
30	ATOM	649	CE2	TYR	84	-0.090	12.739	-6.335	1.00	63.40	1SG 650
	ATOM	650	CZ	TYR	84	-0.346	11.960	-5.236	1.00	63.40	1SG 651
	ATOM	651	OH	TYR	84	0.621	11.848	-4.215	1.00	63.40	1SG 652

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	652	C	TYR	84	-4.198	10.135	-8.716	1.00	63.40	1SG 653
ATOM	653	O	TYR	84	-5.355	10.206	-9.123	1.00	63.40	1SG 654
ATOM	654	N	LYS	85	-3.839	9.209	-7.814	1.00	72.58	1SG 655
ATOM	655	CA	LYS	85	-4.856	8.315	-7.354	1.00	72.58	1SG 656
ATOM	656	CB	LYS	85	-4.428	6.838	-7.365	1.00	72.58	1SG 657
ATOM	657	CG	LYS	85	-4.269	6.258	-8.774	1.00	72.58	1SG 658
ATOM	658	CD	LYS	85	-5.537	6.382	-9.624	1.00	72.58	1SG 659
ATOM	659	CE	LYS	85	-5.496	5.585	-10.931	1.00	72.58	1SG 660
ATOM	660	NZ	LYS	85	-4.450	6.118	-11.832	1.00	72.58	1SG 661
ATOM	661	C	LYS	85	-5.173	8.680	-5.949	1.00	72.58	1SG 662
ATOM	662	O	LYS	85	-4.414	9.381	-5.284	1.00	72.58	1SG 663
ATOM	663	N	GLY	86	-6.293	8.160	-5.428	1.00	79.59	1SG 664
ATOM	664	CA	GLY	86	-6.600	8.530	-4.086	1.00	79.59	1SG 665
ATOM	665	C	GLY	86	-7.612	7.565	-3.575	1.00	79.59	1SG 666
ATOM	666	O	GLY	86	-8.562	7.929	-2.893	1.00	79.59	1SG 667
ATOM	667	N	GLY	87	-7.281	6.269	-3.714	1.00	82.84	1SG 668
ATOM	668	CA	GLY	87	-8.143	5.212	-3.282	1.00	82.84	1SG 669
ATOM	669	C	GLY	87	-7.862	3.982	-4.104	1.00	82.84	1SG 670
ATOM	670	O	GLY	87	-7.291	3.040	-3.577	1.00	82.84	1SG 671
ATOM	671	N	GLU	88	-8.157	3.975	-5.411	1.00	81.60	1SG 672
ATOM	672	CA	GLU	88	-7.980	2.864	-6.329	1.00	81.60	1SG 673
ATOM	673	CB	GLU	88	-6.703	2.910	-7.168	1.00	81.60	1SG 674
ATOM	674	CG	GLU	88	-6.653	4.106	-8.118	1.00	81.60	1SG 675
ATOM	675	CD	GLU	88	-7.898	4.060	-8.991	1.00	81.60	1SG 676
ATOM	676	OE1	GLU	88	-8.126	3.017	-9.660	1.00	81.60	1SG 677
ATOM	677	OE2	GLU	88	-8.645	5.075	-8.994	1.00	81.60	1SG 678
ATOM	678	C	GLU	88	-8.225	1.460	-5.787	1.00	81.60	1SG 679
ATOM	679	O	GLU	88	-8.663	1.254	-4.657	1.00	81.60	1SG 680
ATOM	680	N	GLU	89	-8.013	0.431	-6.656	1.00	75.52	1SG 681
ATOM	681	CA	GLU	89	-8.298	-0.958	-6.340	1.00	75.52	1SG 682

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	682	CB	GLU	89	-9.371	-1.608	-7.238	1.00	75.52	1SG 683
	ATOM	683	CG	GLU	89	-10.804	-1.270	-6.813	1.00	75.52	1SG 684
5	ATOM	684	CD	GLU	89	-11.217	-2.282	-5.749	1.00	75.52	1SG 685
	ATOM	685	OE1	GLU	89	-10.499	-3.307	-5.600	1.00	75.52	1SG 686
	ATOM	686	OE2	GLU	89	-12.257	-2.049	-5.076	1.00	75.52	1SG 687
	ATOM	687	C	GLU	89	-7.054	-1.811	-6.426	1.00	75.52	1SG 688
	ATOM	688	O	GLU	89	-6.055	-1.435	-7.019	1.00	75.52	1SG 689
10	ATOM	689	N	ALA	90	-7.066	-3.024	-5.844	1.00	73.23	1SG 690
	ATOM	690	CA	ALA	90	-5.868	-3.821	-5.812	1.00	73.23	1SG 691
	ATOM	691	CB	ALA	90	-6.075	-5.191	-5.142	1.00	73.23	1SG 692
	ATOM	692	C	ALA	90	-5.299	-4.073	-7.188	1.00	73.23	1SG 693
	ATOM	693	O	ALA	90	-5.976	-4.582	-8.081	1.00	73.23	1SG 694
15	ATOM	694	N	THR	91	-4.002	-3.715	-7.358	1.00	76.69	1SG 695
	ATOM	695	CA	THR	91	-3.218	-3.923	-8.554	1.00	76.69	1SG 696
	ATOM	696	CB	THR	91	-3.348	-2.825	-9.565	1.00	76.69	1SG 697
	ATOM	697	OG1	THR	91	-2.778	-3.225	-10.802	1.00	76.69	1SG 698
	ATOM	698	CG2	THR	91	-2.613	-1.588	-9.024	1.00	76.69	1SG 699
20	ATOM	699	C	THR	91	-1.789	-3.897	-8.081	1.00	76.69	1SG 700
	ATOM	700	O	THR	91	-1.546	-3.688	-6.899	1.00	76.69	1SG 701
	ATOM	701	N	ARG	92	-0.779	-4.097	-8.957	1.00	83.50	1SG 702
	ATOM	702	CA	ARG	92	0.550	-4.076	-8.404	1.00	83.50	1SG 703
	ATOM	703	CB	ARG	92	1.571	-4.950	-9.162	1.00	83.50	1SG 704
25	ATOM	704	CG	ARG	92	1.360	-6.457	-8.988	1.00	83.50	1SG 705
	ATOM	705	CD	ARG	92	2.509	-7.298	-9.553	1.00	83.50	1SG 706
	ATOM	706	NE	ARG	92	2.189	-8.736	-9.321	1.00	83.50	1SG 707
	ATOM	707	CZ	ARG	92	2.507	-9.327	-8.133	1.00	83.50	1SG 708
	ATOM	708	NH1	ARG	92	3.084	-8.594	-7.135	1.00	83.50	1SG 709
30	ATOM	709	NH2	ARG	92	2.253	-10.654	-7.938	1.00	83.50	1SG 710
	ATOM	710	C	ARG	92	1.098	-2.673	-8.385	1.00	83.50	1SG 711
	ATOM	711	O	ARG	92	2.048	-2.377	-9.102	1.00	83.50	1SG 712

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	712	N	PHE	93	0.386	-1.709	-7.769	1.00	91.53	1SG 713
ATOM	713	CA	PHE	93	0.882	-0.395	-7.407	1.00	91.53	1SG 714
ATOM	714	CB	PHE	93	0.116	0.754	-8.068	1.00	91.53	1SG 715
ATOM	715	CG	PHE	93	0.776	1.072	-9.369	1.00	91.53	1SG 716
ATOM	716	CD1	PHE	93	0.455	0.407	-10.530	1.00	91.53	1SG 717
ATOM	717	CD2	PHE	93	1.743	2.055	-9.415	1.00	91.53	1SG 718
ATOM	718	CE1	PHE	93	1.084	0.726	-11.713	1.00	91.53	1SG 719
ATOM	719	CE2	PHE	93	2.373	2.377	-10.595	1.00	91.53	1SG 720
ATOM	720	CZ	PHE	93	2.043	1.711	-11.750	1.00	91.53	1SG 721
ATOM	721	C	PHE	93	0.997	-0.098	-5.918	1.00	91.53	1SG 722
ATOM	722	O	PHE	93	1.751	0.783	-5.509	1.00	91.53	1SG 723
ATOM	723	N	THR	94	0.159	-0.778	-5.101	1.00	92.25	1SG 724
ATOM	724	CA	THR	94	-0.205	-0.526	-3.719	1.00	92.25	1SG 725
ATOM	725	CB	THR	94	-1.574	-1.115	-3.551	1.00	92.25	1SG 726
ATOM	726	OG1	THR	94	-1.700	-1.825	-2.327	1.00	92.25	1SG 727
ATOM	727	CG2	THR	94	-1.859	-2.036	-4.742	1.00	92.25	1SG 728
ATOM	728	C	THR	94	0.670	-1.156	-2.666	1.00	92.25	1SG 729
ATOM	729	O	THR	94	1.484	-2.031	-2.950	1.00	92.25	1SG 730
ATOM	730	N	PHE	95	0.508	-0.688	-1.392	1.00	85.34	1SG 731
ATOM	731	CA	PHE	95	1.202	-1.286	-0.270	1.00	85.34	1SG 732
ATOM	732	CB	PHE	95	2.422	-0.481	0.215	1.00	85.34	1SG 733
ATOM	733	CG	PHE	95	3.449	-0.351	-0.854	1.00	85.34	1SG 734
ATOM	734	CD1	PHE	95	4.238	-1.418	-1.215	1.00	85.34	1SG 735
ATOM	735	CD2	PHE	95	3.640	0.864	-1.470	1.00	85.34	1SG 736
ATOM	736	CE1	PHE	95	5.190	-1.277	-2.197	1.00	85.34	1SG 737
ATOM	737	CE2	PHE	95	4.590	1.012	-2.451	1.00	85.34	1SG 738
ATOM	738	CZ	PHE	95	5.366	-0.062	-2.816	1.00	85.34	1SG 739
ATOM	739	C	PHE	95	0.297	-1.287	0.936	1.00	85.34	1SG 740
ATOM	740	O	PHE	95	-0.435	-0.326	1.164	1.00	85.34	1SG 741
ATOM	741	N	PHE	96	0.299	-2.353	1.770	1.00	74.79	1SG 742

	Atom		Amino Acid		X	Y	Z	Occup.	B Factor	
	No.	Name	SC	No.						
	ATOM	742 CA	PHE	96	-0.453	-2.149	2.972	1.00	74.79	1SG 743
	ATOM	743 CB	PHE	96	-1.538	-3.186	3.376	1.00	74.79	1SG 744
5	ATOM	744 CG	PHE	96	-1.065	-4.504	3.893	1.00	74.79	1SG 745
	ATOM	745 CD1	PHE	96	-0.376	-4.592	5.083	1.00	74.79	1SG 746
	ATOM	746 CD2	PHE	96	-1.389	-5.670	3.234	1.00	74.79	1SG 747
	ATOM	747 CE1	PHE	96	0.035	-5.809	5.573	1.00	74.79	1SG 748
	ATOM	748 CE2	PHE	96	-0.985	-6.891	3.724	1.00	74.79	1SG 749
10	ATOM	749 CZ	PHE	96	-0.265	-6.965	4.892	1.00	74.79	1SG 750
	ATOM	750 C	PHE	96	0.555	-1.970	4.050	1.00	74.79	1SG 751
	ATOM	751 O	PHE	96	1.472	-2.769	4.212	1.00	74.79	1SG 752
	ATOM	752 N	GLN	97	0.423	-0.863	4.793	1.00	62.31	1SG 753
	ATOM	753 CA	GLN	97	1.368	-0.499	5.799	1.00	62.31	1SG 754
15	ATOM	754 CB	GLN	97	1.438	1.034	5.915	1.00	62.31	1SG 755
	ATOM	755 CG	GLN	97	2.404	1.607	6.950	1.00	62.31	1SG 756
	ATOM	756 CD	GLN	97	2.263	3.123	6.862	1.00	62.31	1SG 757
	ATOM	757 OE1	GLN	97	3.156	3.833	6.402	1.00	62.31	1SG 758
	ATOM	758 NE2	GLN	97	1.080	3.633	7.299	1.00	62.31	1SG 759
20	ATOM	759 C	GLN	97	0.849	-1.039	7.085	1.00	62.31	1SG 760
	ATOM	760 O	GLN	97	-0.081	-0.478	7.658	1.00	62.31	1SG 761
	ATOM	761 N	SER	98	1.448	-2.142	7.582	1.00	50.81	1SG 762
	ATOM	762 CA	SER	98	0.973	-2.712	8.809	1.00	50.81	1SG 763
	ATOM	763 CB	SER	98	1.105	-4.243	8.844	1.00	50.81	1SG 764
25	ATOM	764 OG	SER	98	0.624	-4.754	10.077	1.00	50.81	1SG 765
	ATOM	765 C	SER	98	1.816	-2.168	9.916	1.00	50.81	1SG 766
	ATOM	766 O	SER	98	2.841	-2.743	10.271	1.00	50.81	1SG 767
	ATOM	767 N	SER	99	1.372	-1.061	10.537	1.00	43.31	1SG 768
	ATOM	768 CA	SER	99	2.198	-0.486	11.558	1.00	43.31	1SG 769
30	ATOM	769 CB	SER	99	2.194	1.053	11.550	1.00	43.31	1SG 770
	ATOM	770 OG	SER	99	3.032	1.549	12.584	1.00	43.31	1SG 771
	ATOM	771 C	SER	99	1.694	-0.930	12.886	1.00	43.31	1SG 772

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	772 O	SER	99	0.573	-0.624	13.289	1.00	43.31	1SG 773
	ATOM	773 N	SER	100	2.541	-1.676	13.616	1.00	37.37	1SG 774
5	ATOM	774 CA	SER	100	2.133	-2.108	14.913	1.00	37.37	1SG 775
	ATOM	775 CB	SER	100	2.444	-3.593	15.164	1.00	37.37	1SG 776
	ATOM	776 OG	SER	100	3.826	-3.845	14.952	1.00	37.37	1SG 777
	ATOM	777 C	SER	100	2.899	-1.286	15.889	1.00	37.37	1SG 778
	ATOM	778 O	SER	100	3.984	-1.665	16.326	1.00	37.37	1SG 779
10	ATOM	779 N	GLY	101	2.323	-0.141	16.294	1.00	35.21	1SG 780
	ATOM	780 CA	GLY	101	3.036	0.712	17.195	1.00	35.21	1SG 781
	ATOM	781 C	GLY	101	4.100	1.425	16.410	1.00	35.21	1SG 782
	ATOM	782 O	GLY	101	3.823	2.034	15.378	1.00	35.21	1SG 783
	ATOM	783 N	SER	102	5.349	1.396	16.921	1.00	38.31	1SG 784
15	ATOM	784 CA	SER	102	6.464	2.092	16.332	1.00	38.31	1SG 785
	ATOM	785 CB	SER	102	7.691	2.209	17.260	1.00	38.31	1SG 786
	ATOM	786 OG	SER	102	7.387	3.034	18.374	1.00	38.31	1SG 787
	ATOM	787 C	SER	102	6.976	1.487	15.044	1.00	38.31	1SG 788
	ATOM	788 O	SER	102	7.559	2.217	14.245	1.00	38.31	1SG 789
20	ATOM	789 N	ALA	103	6.814	0.166	14.790	1.00	43.59	1SG 790
	ATOM	790 CA	ALA	103	7.451	-0.404	13.617	1.00	43.59	1SG 791
	ATOM	791 CB	ALA	103	8.435	-1.540	13.956	1.00	43.59	1SG 792
	ATOM	792 C	ALA	103	6.438	-0.971	12.655	1.00	43.59	1SG 793
	ATOM	793 O	ALA	103	5.323	-1.302	13.052	1.00	43.59	1SG 794
25	ATOM	794 N	PHE	104	6.801	-1.090	11.344	1.00	52.49	1SG 795
	ATOM	795 CA	PHE	104	5.834	-1.608	10.400	1.00	52.49	1SG 796
	ATOM	796 CB	PHE	104	5.040	-0.525	9.656	1.00	52.49	1SG 797
	ATOM	797 CG	PHE	104	5.867	0.122	8.604	1.00	52.49	1SG 798
	ATOM	798 CD1	PHE	104	6.978	0.858	8.936	1.00	52.49	1SG 799
30	ATOM	799 CD2	PHE	104	5.536	-0.039	7.277	1.00	52.49	1SG 800
	ATOM	800 CE1	PHE	104	7.728	1.454	7.951	1.00	52.49	1SG 801
	ATOM	801 CE2	PHE	104	6.281	0.559	6.291	1.00	52.49	1SG 802

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	802	CZ	PHE	104	7.381	1.309	6.628	1.00	52.49	1SG 803
	ATOM	803	C	PHE	104	6.460	-2.527	9.377	1.00	52.49	1SG 804
5	ATOM	804	O	PHE	104	7.613	-2.936	9.507	1.00	52.49	1SG 805
	ATOM	805	N	ARG	105	5.644	-2.909	8.354	1.00	61.03	1SG 806
	ATOM	806	CA	ARG	105	5.980	-3.832	7.293	1.00	61.03	1SG 807
	ATOM	807	CB	ARG	105	5.433	-5.232	7.602	1.00	61.03	1SG 808
	ATOM	808	CG	ARG	105	5.822	-5.729	8.997	1.00	61.03	1SG 809
10	ATOM	809	CD	ARG	105	5.102	-7.013	9.405	1.00	61.03	1SG 810
	ATOM	810	NE	ARG	105	5.506	-7.326	10.805	1.00	61.03	1SG 811
	ATOM	811	CZ	ARG	105	6.100	-8.519	11.101	1.00	61.03	1SG 812
	ATOM	812	NH1	ARG	105	6.333	-9.437	10.117	1.00	61.03	1SG 813
	ATOM	813	NH2	ARG	105	6.458	-8.796	12.388	1.00	61.03	1SG 814
15	ATOM	814	C	ARG	105	5.272	-3.372	6.021	1.00	61.03	1SG 815
	ATOM	815	O	ARG	105	4.242	-2.701	6.088	1.00	61.03	1SG 816
	ATOM	816	N	LEU	106	5.792	-3.713	4.810	1.00	71.08	1SG 817
	ATOM	817	CA	LEU	106	5.107	-3.275	3.606	1.00	71.08	1SG 818
	ATOM	818	CB	LEU	106	5.972	-2.446	2.634	1.00	71.08	1SG 819
20	ATOM	819	CG	LEU	106	6.471	-1.092	3.162	1.00	71.08	1SG 820
	ATOM	820	CD2	LEU	106	7.461	-1.285	4.314	1.00	71.08	1SG 821
	ATOM	821	CD1	LEU	106	5.308	-0.145	3.491	1.00	71.08	1SG 822
	ATOM	822	C	LEU	106	4.592	-4.393	2.722	1.00	71.08	1SG 823
	ATOM	823	O	LEU	106	5.344	-5.031	1.990	1.00	71.08	1SG 824
25	ATOM	824	N	GLU	107	3.264	-4.613	2.680	1.00	80.65	1SG 825
	ATOM	825	CA	GLU	107	2.734	-5.598	1.766	1.00	80.65	1SG 826
	ATOM	826	CB	GLU	107	1.306	-6.036	2.098	1.00	80.65	1SG 827
	ATOM	827	CG	GLU	107	0.903	-7.366	1.457	1.00	80.65	1SG 828
	ATOM	828	CD	GLU	107	1.626	-8.496	2.187	1.00	80.65	1SG 829
30	ATOM	829	OE1	GLU	107	2.414	-8.199	3.125	1.00	80.65	1SG 830
	ATOM	830	OE2	GLU	107	1.397	-9.677	1.813	1.00	80.65	1SG 831
	ATOM	831	C	GLU	107	2.737	-4.975	0.386	1.00	80.65	1SG 832

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	832	O	GLU	107	2.674	-3.752	0.260	1.00	80.65	1SG 833
ATOM	833	N	ALA	108	2.816	-5.801	-0.690	1.00	85.77	1SG 834
ATOM	834	CA	ALA	108	2.943	-5.291	-2.042	1.00	85.77	1SG 835
ATOM	835	CB	ALA	108	4.261	-5.704	-2.721	1.00	85.77	1SG 836
ATOM	836	C	ALA	108	1.817	-5.779	-2.907	1.00	85.77	1SG 837
ATOM	837	O	ALA	108	1.309	-6.879	-2.700	1.00	85.77	1SG 838
ATOM	838	N	ALA	109	1.421	-4.968	-3.923	1.00	87.63	1SG 839
ATOM	839	CA	ALA	109	0.192	-5.226	-4.629	1.00	87.63	1SG 840
ATOM	840	CB	ALA	109	-0.072	-6.686	-5.043	1.00	87.63	1SG 841
ATOM	841	C	ALA	109	-0.682	-4.796	-3.518	1.00	87.63	1SG 842
ATOM	842	O	ALA	109	-0.260	-3.864	-2.853	1.00	87.63	1SG 843
ATOM	843	N	ALA	110	-1.960	-5.155	-3.346	1.00	80.92	1SG 844
ATOM	844	CA	ALA	110	-2.367	-4.822	-2.001	1.00	80.92	1SG 845
ATOM	845	CB	ALA	110	-3.788	-4.244	-1.938	1.00	80.92	1SG 846
ATOM	846	C	ALA	110	-2.329	-5.995	-1.047	1.00	80.92	1SG 847
ATOM	847	O	ALA	110	-1.480	-6.110	-0.163	1.00	80.92	1SG 848
ATOM	848	N	TRP	111	-3.319	-6.916	-1.245	1.00	69.81	1SG 849
ATOM	849	CA	TRP	111	-3.479	-8.105	-0.443	1.00	69.81	1SG 850
ATOM	850	CB	TRP	111	-4.946	-8.567	-0.288	1.00	69.81	1SG 851
ATOM	851	CG	TRP	111	-5.836	-7.555	0.392	1.00	69.81	1SG 852
ATOM	852	CD2	TRP	111	-6.624	-6.588	-0.316	1.00	69.81	1SG 853
ATOM	853	CD1	TRP	111	-6.080	-7.355	1.719	1.00	69.81	1SG 854
ATOM	854	NE1	TRP	111	-6.969	-6.319	1.882	1.00	69.81	1SG 855
ATOM	855	CE2	TRP	111	-7.314	-5.840	0.636	1.00	69.81	1SG 856
ATOM	856	CE3	TRP	111	-6.766	-6.348	-1.654	1.00	69.81	1SG 857
ATOM	857	CZ2	TRP	111	-8.156	-4.832	0.265	1.00	69.81	1SG 858
ATOM	858	CZ3	TRP	111	-7.615	-5.330	-2.026	1.00	69.81	1SG 859
ATOM	859	CH2	TRP	111	-8.297	-4.586	-1.083	1.00	69.81	1SG 860
ATOM	860	C	TRP	111	-2.609	-9.224	-0.949	1.00	69.81	1SG 861
ATOM	861	O	TRP	111	-1.799	-9.759	-0.194	1.00	69.81	1SG 862

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	862	N	PRO	112	-2.728	-9.603	-2.212	1.00	62.43	1SG 863
	ATOM	863	CA	PRO	112	-1.865	-10.612	-2.763	1.00	62.43	1SG 864
5	ATOM	864	CD	PRO	112	-3.976	-9.518	-2.960	1.00	62.43	1SG 865
	ATOM	865	CB	PRO	112	-2.652	-11.309	-3.872	1.00	62.43	1SG 866
	ATOM	866	CG	PRO	112	-3.722	-10.282	-4.268	1.00	62.43	1SG 867
	ATOM	867	C	PRO	112	-0.692	-9.863	-3.289	1.00	62.43	1SG 868
	ATOM	868	O	PRO	112	-0.720	-8.634	-3.238	1.00	62.43	1SG 869
10	ATOM	869	N	GLY	113	0.349	-10.556	-3.793	1.00	62.37	1SG 870
	ATOM	870	CA	GLY	113	1.428	-9.790	-4.339	1.00	62.37	1SG 871
	ATOM	871	C	GLY	113	2.736	-10.297	-3.803	1.00	62.37	1SG 872
	ATOM	872	O	GLY	113	3.147	-11.409	-4.126	1.00	62.37	1SG 873
	ATOM	873	N	TRP	114	3.446	-9.452	-3.012	1.00	67.98	1SG 874
15	ATOM	874	CA	TRP	114	4.724	-9.793	-2.437	1.00	67.98	1SG 875
	ATOM	875	CB	TRP	114	5.914	-9.196	-3.203	1.00	67.98	1SG 876
	ATOM	876	CG	TRP	114	6.075	-9.845	-4.556	1.00	67.98	1SG 877
	ATOM	877	CD2	TRP	114	7.053	-10.850	-4.866	1.00	67.98	1SG 878
	ATOM	878	CD1	TRP	114	5.355	-9.641	-5.697	1.00	67.98	1SG 879
20	ATOM	879	NE1	TRP	114	5.810	-10.470	-6.691	1.00	67.98	1SG 880
	ATOM	880	CE2	TRP	114	6.858	-11.217	-6.198	1.00	67.98	1SG 881
	ATOM	881	CE3	TRP	114	8.032	-11.425	-4.104	1.00	67.98	1SG 882
	ATOM	882	CZ2	TRP	114	7.640	-12.165	-6.792	1.00	67.98	1SG 883
	ATOM	883	CZ3	TRP	114	8.820	-12.380	-4.709	1.00	67.98	1SG 884
25	ATOM	884	CH2	TRP	114	8.627	-12.743	-6.026	1.00	67.98	1SG 885
	ATOM	885	C	TRP	114	4.774	-9.341	-0.991	1.00	67.98	1SG 886
	ATOM	886	O	TRP	114	3.866	-8.644	-0.535	1.00	67.98	1SG 887
	ATOM	887	N	PHE	115	5.840	-9.745	-0.234	1.00	75.12	1SG 888
	ATOM	888	CA	PHE	115	5.900	-9.542	1.201	1.00	75.12	1SG 889
30	ATOM	889	CB	PHE	115	6.118	-10.851	1.984	1.00	75.12	1SG 890
	ATOM	890	CG	PHE	115	4.938	-11.737	1.781	1.00	75.12	1SG 891
	ATOM	891	CD1	PHE	115	3.809	-11.586	2.549	1.00	75.12	1SG 892

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	892	CD2	PHE	115	4.965	-12.726	0.825	1.00	75.12	1SG 893
	ATOM	893	CE1	PHE	115	2.722	-12.406	2.363	1.00	75.12	1SG 894
5	ATOM	894	CE2	PHE	115	3.879	-13.549	0.635	1.00	75.12	1SG 895
	ATOM	895	CZ	PHE	115	2.753	-13.390	1.405	1.00	75.12	1SG 896
	ATOM	896	C	PHE	115	7.005	-8.617	1.687	1.00	75.12	1SG 897
	ATOM	897	O	PHE	115	8.185	-8.745	1.381	1.00	75.12	1SG 898
	ATOM	898	N	LEU	116	6.589	-7.733	2.604	1.00	79.62	1SG 899
10	ATOM	899	CA	LEU	116	7.128	-6.614	3.344	1.00	79.62	1SG 900
	ATOM	900	CB	LEU	116	6.186	-6.453	4.520	1.00	79.62	1SG 901
	ATOM	901	CG	LEU	116	5.684	-7.837	4.981	1.00	79.62	1SG 902
	ATOM	902	CD2	LEU	116	4.748	-7.722	6.181	1.00	79.62	1SG 903
	ATOM	903	CD1	LEU	116	6.835	-8.808	5.266	1.00	79.62	1SG 904
15	ATOM	904	C	LEU	116	8.481	-6.700	4.035	1.00	79.62	1SG 905
	ATOM	905	O	LEU	116	8.505	-6.311	5.200	1.00	79.62	1SG 906
	ATOM	906	N	CYS	117	9.637	-7.046	3.414	1.00	73.41	1SG 907
	ATOM	907	CA	CYS	117	10.854	-7.122	4.216	1.00	73.41	1SG 908
	ATOM	908	CB	CYS	117	11.489	-8.525	4.188	1.00	73.41	1SG 909
20	ATOM	909	SG	CYS	117	10.392	-9.804	4.874	1.00	73.41	1SG 910
	ATOM	910	C	CYS	117	11.925	-6.146	3.754	1.00	73.41	1SG 911
	ATOM	911	O	CYS	117	11.660	-5.250	2.953	1.00	73.41	1SG 912
	ATOM	912	N	GLY	118	13.169	-6.282	4.305	1.00	66.61	1SG 913
	ATOM	913	CA	GLY	118	14.312	-5.456	3.968	1.00	66.61	1SG 914
25	ATOM	914	C	GLY	118	15.571	-6.125	4.494	1.00	66.61	1SG 915
	ATOM	915	O	GLY	118	15.574	-6.721	5.568	1.00	66.61	1SG 916
	ATOM	916	N	PRO	119	16.642	-6.042	3.738	1.00	63.36	1SG 917
	ATOM	917	CA	PRO	119	17.904	-6.605	4.165	1.00	63.36	1SG 918
	ATOM	918	CD	PRO	119	16.408	-6.432	2.368	1.00	63.36	1SG 919
30	ATOM	919	CB	PRO	119	18.784	-6.602	2.925	1.00	63.36	1SG 920
	ATOM	920	CG	PRO	119	17.758	-6.941	1.826	1.00	63.36	1SG 921
	ATOM	921	C	PRO	119	18.522	-6.093	5.431	1.00	63.36	1SG 922

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	922	O	PRO	119	18.339	-4.932	5.781	1.00	63.36	1SG 923
ATOM	923	N	ALA	120	19.201	-7.003	6.165	1.00	59.66	1SG 924
ATOM	924	CA	ALA	120	19.788	-6.772	7.458	1.00	59.66	1SG 925
ATOM	925	CB	ALA	120	20.233	-8.078	8.139	1.00	59.66	1SG 926
ATOM	926	C	ALA	120	20.980	-5.841	7.507	1.00	59.66	1SG 927
ATOM	927	O	ALA	120	21.012	-4.965	8.368	1.00	59.66	1SG 928
ATOM	928	N	GLU	121	21.987	-5.975	6.611	1.00	62.17	1SG 929
ATOM	929	CA	GLU	121	23.212	-5.236	6.838	1.00	62.17	1SG 930
ATOM	930	CB	GLU	121	24.431	-6.175	6.910	1.00	62.17	1SG 931
ATOM	931	CG	GLU	121	25.562	-5.661	7.797	1.00	62.17	1SG 932
ATOM	932	CD	GLU	121	25.087	-5.778	9.242	1.00	62.17	1SG 933
ATOM	933	OE1	GLU	121	24.274	-6.696	9.535	1.00	62.17	1SG 934
ATOM	934	OE2	GLU	121	25.532	-4.943	10.073	1.00	62.17	1SG 935
ATOM	935	C	GLU	121	23.442	-4.222	5.746	1.00	62.17	1SG 936
ATOM	936	O	GLU	121	22.727	-4.214	4.745	1.00	62.17	1SG 937
ATOM	937	N	PRO	122	24.392	-3.319	5.931	1.00	63.98	1SG 938
ATOM	938	CA	PRO	122	24.632	-2.311	4.927	1.00	63.98	1SG 939
ATOM	939	CD	PRO	122	24.701	-2.813	7.256	1.00	63.98	1SG 940
ATOM	940	CB	PRO	122	25.479	-1.223	5.595	1.00	63.98	1SG 941
ATOM	941	CG	PRO	122	25.814	-1.788	6.992	1.00	63.98	1SG 942
ATOM	942	C	PRO	122	25.142	-2.803	3.616	1.00	63.98	1SG 943
ATOM	943	O	PRO	122	26.359	-2.872	3.456	1.00	63.98	1SG 944
ATOM	944	N	GLN	123	24.229	-3.037	2.644	1.00	62.66	1SG 945
ATOM	945	CA	GLN	123	24.578	-3.584	1.363	1.00	62.66	1SG 946
ATOM	946	CB	GLN	123	24.968	-5.073	1.449	1.00	62.66	1SG 947
ATOM	947	CG	GLN	123	26.243	-5.331	2.257	1.00	62.66	1SG 948
ATOM	948	CD	GLN	123	26.356	-6.820	2.544	1.00	62.66	1SG 949
ATOM	949	OE1	GLN	123	25.369	-7.554	2.534	1.00	62.66	1SG 950
ATOM	950	NE2	GLN	123	27.604	-7.283	2.826	1.00	62.66	1SG 951
ATOM	951	C	GLN	123	23.366	-3.515	0.465	1.00	62.66	1SG 952

	Atom	No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	952	O	GLN	123	23.283	-2.648	-0.404	1.00	62.66	1SG 953
	ATOM	953	N	GLN	124	22.417	-4.470	0.632	1.00	62.66	1SG 954
5	ATOM	954	CA	GLN	124	21.243	-4.591	-0.205	1.00	62.66	1SG 955
	ATOM	955	CB	GLN	124	20.703	-6.025	-0.329	1.00	62.66	1SG 956
	ATOM	956	CG	GLN	124	21.649	-6.978	-1.061	1.00	62.66	1SG 957
	ATOM	957	CD	GLN	124	20.854	-8.233	-1.384	1.00	62.66	1SG 958
	ATOM	958	OE1	GLN	124	19.643	-8.168	-1.585	1.00	62.66	1SG 959
10	ATOM	959	NE2	GLN	124	21.544	-9.402	-1.442	1.00	62.66	1SG 960
	ATOM	960	C	GLN	124	20.102	-3.707	0.250	1.00	62.66	1SG 961
	ATOM	961	O	GLN	124	20.093	-3.156	1.352	1.00	62.66	1SG 962
	ATOM	962	N	PRO	125	19.158	-3.548	-0.661	1.00	62.42	1SG 963
	ATOM	963	CA	PRO	125	17.985	-2.698	-0.494	1.00	62.42	1SG 964
15	ATOM	964	CD	PRO	125	19.553	-3.620	-2.060	1.00	62.42	1SG 965
	ATOM	965	CB	PRO	125	17.688	-2.107	-1.872	1.00	62.42	1SG 966
	ATOM	966	CG	PRO	125	18.355	-3.080	-2.853	1.00	62.42	1SG 967
	ATOM	967	C	PRO	125	16.746	-3.330	0.075	1.00	62.42	1SG 968
	ATOM	968	O	PRO	125	16.795	-4.495	0.439	1.00	62.42	1SG 969
20	ATOM	969	N	VAL	126	15.621	-2.569	0.130	1.00	63.03	1SG 970
	ATOM	970	CA	VAL	126	14.329	-3.006	0.618	1.00	63.03	1SG 971
	ATOM	971	CB	VAL	126	13.319	-1.890	0.647	1.00	63.03	1SG 972
	ATOM	972	CG1	VAL	126	13.203	-1.283	-0.764	1.00	63.03	1SG 973
	ATOM	973	CG2	VAL	126	11.990	-2.433	1.199	1.00	63.03	1SG 974
25	ATOM	974	C	VAL	126	13.808	-4.122	-0.255	1.00	63.03	1SG 975
	ATOM	975	O	VAL	126	13.983	-4.108	-1.474	1.00	63.03	1SG 976
	ATOM	976	N	GLN	127	13.156	-5.146	0.353	1.00	63.16	1SG 977
	ATOM	977	CA	GLN	127	12.754	-6.261	-0.464	1.00	63.16	1SG 978
	ATOM	978	CB	GLN	127	13.705	-7.464	-0.329	1.00	63.16	1SG 979
30	ATOM	979	CG	GLN	127	13.396	-8.616	-1.283	1.00	63.16	1SG 980
	ATOM	980	CD	GLN	127	14.588	-9.562	-1.248	1.00	63.16	1SG 981
	ATOM	981	OE1	GLN	127	15.722	-9.152	-1.490	1.00	63.16	1SG 982

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	982	NE2	GLN	127	14.330	-10.859	-0.934	1.00	63.16	1SG 983
ATOM	983	C	GLN	127	11.354	-6.734	-0.180	1.00	63.16	1SG 984
ATOM	984	O	GLN	127	10.978	-7.006	0.960	1.00	63.16	1SG 985
ATOM	985	N	LEU	128	10.555	-6.862	-1.264	1.00	62.34	1SG 986
ATOM	986	CA	LEU	128	9.216	-7.389	-1.248	1.00	62.34	1SG 987
ATOM	987	CB	LEU	128	8.348	-6.718	-2.326	1.00	62.34	1SG 988
ATOM	988	CG	LEU	128	8.284	-5.182	-2.195	1.00	62.34	1SG 989
ATOM	989	CD2	LEU	128	7.831	-4.749	-0.793	1.00	62.34	1SG 990
ATOM	990	CD1	LEU	128	7.423	-4.561	-3.304	1.00	62.34	1SG 991
ATOM	991	C	LEU	128	9.422	-8.826	-1.660	1.00	62.34	1SG 992
ATOM	992	O	LEU	128	9.557	-9.131	-2.844	1.00	62.34	1SG 993
ATOM	993	N	THR	129	9.464	-9.728	-0.660	1.00	61.72	1SG 994
ATOM	994	CA	THR	129	9.832	-11.116	-0.762	1.00	61.72	1SG 995
ATOM	995	CB	THR	129	10.572	-11.524	0.477	1.00	61.72	1SG 996
ATOM	996	OG1	THR	129	10.953	-12.885	0.419	1.00	61.72	1SG 997
ATOM	997	CG2	THR	129	9.665	-11.263	1.692	1.00	61.72	1SG 998
ATOM	998	C	THR	129	8.666	-12.052	-0.955	1.00	61.72	1SG 999
ATOM	999	O	THR	129	7.499	-11.690	-0.820	1.00	61.72	1SG1000
ATOM	1000	N	LYS	130	8.997	-13.307	-1.341	1.00	60.09	1SG1001
ATOM	1001	CA	LYS	130	8.075	-14.390	-1.569	1.00	60.09	1SG1002
ATOM	1002	CB	LYS	130	8.684	-15.547	-2.380	1.00	60.09	1SG1003
ATOM	1003	CG	LYS	130	7.652	-16.602	-2.792	1.00	60.09	1SG1004
ATOM	1004	CD	LYS	130	8.091	-17.469	-3.977	1.00	60.09	1SG1005
ATOM	1005	CE	LYS	130	8.073	-16.707	-5.308	1.00	60.09	1SG1006
ATOM	1006	NZ	LYS	130	8.478	-17.593	-6.422	1.00	60.09	1SG1007
ATOM	1007	C	LYS	130	7.532	-14.951	-0.286	1.00	60.09	1SG1008
ATOM	1008	O	LYS	130	6.398	-15.425	-0.244	1.00	60.09	1SG1009
ATOM	1009	N	GLU	131	8.333	-14.964	0.797	1.00	60.35	1SG1010
ATOM	1010	CA	GLU	131	7.828	-15.598	1.981	1.00	60.35	1SG1011
ATOM	1011	CB	GLU	131	8.414	-17.007	2.172	1.00	60.35	1SG1012

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	1012	CG	GLU	131	9.943	-17.030	2.105	1.00	60.35	1SG1013
ATOM	1013	CD	GLU	131	10.389	-18.485	2.073	1.00	60.35	1SG1014
ATOM	1014	OE1	GLU	131	9.524	-19.373	2.301	1.00	60.35	1SG1015
ATOM	1015	OE2	GLU	131	11.597	-18.727	1.813	1.00	60.35	1SG1016
ATOM	1016	C	GLU	131	8.134	-14.776	3.197	1.00	60.35	1SG1017
ATOM	1017	O	GLU	131	8.917	-13.827	3.159	1.00	60.35	1SG1018
ATOM	1018	N	SER	132	7.456	-15.127	4.310	1.00	60.94	1SG1019
ATOM	1019	CA	SER	132	7.647	-14.555	5.614	1.00	60.94	1SG1020
ATOM	1020	CB	SER	132	6.392	-14.594	6.505	1.00	60.94	1SG1021
ATOM	1021	OG	SER	132	5.399	-13.719	5.989	1.00	60.94	1SG1022
ATOM	1022	C	SER	132	8.681	-15.454	6.230	1.00	60.94	1SG1023
ATOM	1023	O	SER	132	9.704	-15.707	5.597	1.00	60.94	1SG1024
ATOM	1024	N	GLU	133	8.477	-15.928	7.483	1.00	59.09	1SG1025
ATOM	1025	CA	GLU	133	9.402	-16.875	8.059	1.00	59.09	1SG1026
ATOM	1026	CB	GLU	133	9.774	-18.016	7.090	1.00	59.09	1SG1027
ATOM	1027	CG	GLU	133	8.602	-18.946	6.765	1.00	59.09	1SG1028
ATOM	1028	CD	GLU	133	8.982	-19.777	5.546	1.00	59.09	1SG1029
ATOM	1029	OE1	GLU	133	10.160	-20.219	5.468	1.00	59.09	1SG1030
ATOM	1030	OE2	GLU	133	8.098	-19.969	4.668	1.00	59.09	1SG1031
ATOM	1031	C	GLU	133	10.647	-16.140	8.439	1.00	59.09	1SG1032
ATOM	1032	O	GLU	133	10.774	-14.946	8.174	1.00	59.09	1SG1033
ATOM	1033	N	PRO	134	11.560	-16.810	9.092	1.00	58.29	1SG1034
ATOM	1034	CA	PRO	134	12.814	-16.224	9.467	1.00	58.29	1SG1035
ATOM	1035	CD	PRO	134	11.329	-18.096	9.721	1.00	58.29	1SG1036
ATOM	1036	CB	PRO	134	13.546	-17.284	10.295	1.00	58.29	1SG1037
ATOM	1037	CG	PRO	134	12.749	-18.585	10.055	1.00	58.29	1SG1038
ATOM	1038	C	PRO	134	13.485	-15.806	8.204	1.00	58.29	1SG1039
ATOM	1039	O	PRO	134	13.608	-16.631	7.299	1.00	58.29	1SG1040
ATOM	1040	N	SER	135	13.927	-14.536	8.122	1.00	58.09	1SG1041
ATOM	1041	CA	SER	135	14.472	-14.043	6.894	1.00	58.09	1SG1042

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	1042	CB	SER	135	13.418	-13.831	5.791	1.00	58.09	1SG1043
ATOM	1043	OG	SER	135	12.851	-15.071	5.393	1.00	58.09	1SG1044
ATOM	1044	C	SER	135	15.079	-12.696	7.147	1.00	58.09	1SG1045
ATOM	1045	O	SER	135	16.161	-12.577	7.718	1.00	58.09	1SG1046
ATOM	1046	N	ALA	136	14.377	-11.641	6.680	1.00	60.01	1SG1047
ATOM	1047	CA	ALA	136	14.857	-10.284	6.707	1.00	60.01	1SG1048
ATOM	1048	CB	ALA	136	14.753	-9.576	5.346	1.00	60.01	1SG1049
ATOM	1049	C	ALA	136	14.091	-9.447	7.695	1.00	60.01	1SG1050
ATOM	1050	O	ALA	136	13.290	-9.959	8.476	1.00	60.01	1SG1051
ATOM	1051	N	ARG	137	14.372	-8.116	7.712	1.00	65.19	1SG1052
ATOM	1052	CA	ARG	137	13.759	-7.251	8.687	1.00	65.19	1SG1053
ATOM	1053	CB	ARG	137	14.663	-6.095	9.165	1.00	65.19	1SG1054
ATOM	1054	CG	ARG	137	15.054	-5.086	8.083	1.00	65.19	1SG1055
ATOM	1055	CD	ARG	137	16.029	-4.017	8.583	1.00	65.19	1SG1056
ATOM	1056	NE	ARG	137	16.251	-3.052	7.473	1.00	65.19	1SG1057
ATOM	1057	CZ	ARG	137	15.451	-1.952	7.361	1.00	65.19	1SG1058
ATOM	1058	NH1	ARG	137	14.476	-1.722	8.288	1.00	65.19	1SG1059
ATOM	1059	NH2	ARG	137	15.623	-1.081	6.324	1.00	65.19	1SG1060
ATOM	1060	C	ARG	137	12.483	-6.647	8.178	1.00	65.19	1SG1061
ATOM	1061	O	ARG	137	12.486	-5.730	7.357	1.00	65.19	1SG1062
ATOM	1062	N	THR	138	11.344	-7.155	8.692	1.00	70.42	1SG1063
ATOM	1063	CA	THR	138	10.062	-6.596	8.373	1.00	70.42	1SG1064
ATOM	1064	CB	THR	138	9.075	-7.634	7.898	1.00	70.42	1SG1065
ATOM	1065	OG1	THR	138	7.833	-7.025	7.583	1.00	70.42	1SG1066
ATOM	1066	CG2	THR	138	8.883	-8.719	8.976	1.00	70.42	1SG1067
ATOM	1067	C	THR	138	9.548	-5.977	9.639	1.00	70.42	1SG1068
ATOM	1068	O	THR	138	8.432	-6.249	10.081	1.00	70.42	1SG1069
ATOM	1069	N	LYS	139	10.369	-5.102	10.251	1.00	75.06	1SG1070
ATOM	1070	CA	LYS	139	10.010	-4.426	11.463	1.00	75.06	1SG1071
ATOM	1071	CB	LYS	139	10.275	-5.321	12.680	1.00	75.06	1SG1072

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	1072	CG	LYS 139	11.555	-6.131	12.484	1.00	75.06	1SG1073
	ATOM	1073	CD	LYS 139	11.916	-7.063	13.633	1.00	75.06	1SG1074
	ATOM	1074	CE	LYS 139	12.988	-8.081	13.241	1.00	75.06	1SG1075
	ATOM	1075	NZ	LYS 139	14.190	-7.383	12.733	1.00	75.06	1SG1076
	ATOM	1076	C	LYS 139	10.879	-3.212	11.539	1.00	75.06	1SG1077
	ATOM	1077	O	LYS 139	11.910	-3.212	12.210	1.00	75.06	1SG1078
	ATOM	1078	N	PHE 140	10.475	-2.127	10.850	1.00	75.24	1SG1079
	ATOM	1079	CA	PHE 140	11.255	-0.918	10.860	1.00	75.24	1SG1080
	ATOM	1080	CB	PHE 140	12.050	-0.659	9.566	1.00	75.24	1SG1081
	ATOM	1081	CG	PHE 140	11.191	-0.999	8.400	1.00	75.24	1SG1082
	ATOM	1082	CD1	PHE 140	11.076	-2.319	8.033	1.00	75.24	1SG1083
	ATOM	1083	CD2	PHE 140	10.529	-0.044	7.667	1.00	75.24	1SG1084
	ATOM	1084	CE1	PHE 140	10.307	-2.698	6.961	1.00	75.24	1SG1085
	ATOM	1085	CE2	PHE 140	9.758	-0.419	6.592	1.00	75.24	1SG1086
	ATOM	1086	CZ	PHE 140	9.646	-1.741	6.236	1.00	75.24	1SG1087
	ATOM	1087	C	PHE 140	10.401	0.251	11.249	1.00	75.24	1SG1088
	ATOM	1088	O	PHE 140	9.182	0.216	11.091	1.00	75.24	1SG1089
	ATOM	1089	N	TYR 141	11.011	1.336	11.786	1.00	71.99	1SG1090
	ATOM	1090	CA	TYR 141	10.129	2.331	12.330	1.00	71.99	1SG1091
	ATOM	1091	CB	TYR 141	10.335	2.675	13.823	1.00	71.99	1SG1092
	ATOM	1092	CG	TYR 141	11.540	3.500	14.095	1.00	71.99	1SG1093
	ATOM	1093	CD1	TYR 141	11.440	4.871	14.075	1.00	71.99	1SG1094
	ATOM	1094	CD2	TYR 141	12.747	2.915	14.393	1.00	71.99	1SG1095
	ATOM	1095	CE1	TYR 141	12.535	5.654	14.343	1.00	71.99	1SG1096
	ATOM	1096	CE2	TYR 141	13.848	3.695	14.661	1.00	71.99	1SG1097
	ATOM	1097	CZ	TYR 141	13.740	5.065	14.635	1.00	71.99	1SG1098
	ATOM	1098	OH	TYR 141	14.860	5.874	14.910	1.00	71.99	1SG1099
	ATOM	1099	C	TYR 141	9.996	3.563	11.493	1.00	71.99	1SG1100
	ATOM	1100	O	TYR 141	10.895	3.959	10.752	1.00	71.99	1SG1101
	ATOM	1101	N	PHE 142	8.778	4.146	11.589	1.00	66.71	1SG1102

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	1102	CA	PHE 142	8.258	5.281	10.875	1.00	66.71	1SG1103
	ATOM	1103	CB	PHE 142	6.760	5.515	11.114	1.00	66.71	1SG1104
5	ATOM	1104	CG	PHE 142	5.917	4.446	10.532	1.00	66.71	1SG1105
	ATOM	1105	CD1	PHE 142	5.787	3.237	11.169	1.00	66.71	1SG1106
	ATOM	1106	CD2	PHE 142	5.254	4.665	9.351	1.00	66.71	1SG1107
	ATOM	1107	CE1	PHE 142	4.995	2.258	10.623	1.00	66.71	1SG1108
	ATOM	1108	CE2	PHE 142	4.463	3.690	8.804	1.00	66.71	1SG1109
10	ATOM	1109	CZ	PHE 142	4.333	2.484	9.441	1.00	66.71	1SG1110
	ATOM	1110	C	PHE 142	8.789	6.558	11.441	1.00	66.71	1SG1111
	ATOM	1111	O	PHE 142	8.757	6.768	12.653	1.00	66.71	1SG1112
	ATOM	1112	N	GLU 143	9.234	7.468	10.550	1.00	57.45	1SG1113
	ATOM	1113	CA	GLU 143	9.601	8.798	10.942	1.00	57.45	1SG1114
15	ATOM	1114	CB	GLU 143	11.104	9.128	10.835	1.00	57.45	1SG1115
	ATOM	1115	CG	GLU 143	12.001	8.325	11.780	1.00	57.45	1SG1116
	ATOM	1116	CD	GLU 143	12.523	7.118	11.012	1.00	57.45	1SG1117
	ATOM	1117	OE1	GLU 143	12.818	7.278	9.798	1.00	57.45	1SG1118
	ATOM	1118	OE2	GLU 143	12.648	6.026	11.627	1.00	57.45	1SG1119
20	ATOM	1119	C	GLU 143	8.901	9.692	9.969	1.00	57.45	1SG1120
	ATOM	1120	O	GLU 143	8.922	9.445	8.764	1.00	57.45	1SG1121
	ATOM	1121	N	GLN 144	8.240	10.756	10.464	1.00	49.90	1SG1122
	ATOM	1122	CA	GLN 144	7.558	11.622	9.548	1.00	49.90	1SG1123
	ATOM	1123	CB	GLN 144	6.056	11.771	9.857	1.00	49.90	1SG1124
25	ATOM	1124	CG	GLN 144	5.282	12.512	8.763	1.00	49.90	1SG1125
	ATOM	1125	CD	GLN 144	3.812	12.557	9.159	1.00	49.90	1SG1126
	ATOM	1126	OE1	GLN 144	2.974	13.057	8.411	1.00	49.90	1SG1127
	ATOM	1127	NE2	GLN 144	3.487	12.020	10.366	1.00	49.90	1SG1128
	ATOM	1128	C	GLN 144	8.213	12.992	9.651	1.00	49.90	1SG1129
30	ATOM	1129	O	GLN 144	9.430	13.084	9.335	1.00	49.90	1SG1130
	ATOM	1130	OXT	GLN 144	7.512	13.961	10.048	1.00	49.90	1SG1131

Table III show protein database coordinates for a IL-1 Hy2 structural models generated by the Protein Data Bank GeneAtlas™ Program (MSI) using the three-dimensional structure of IL-1 β as a template.

Table III:

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	1	N	PRO	1	38.534	-21.658	6.515	1.00	33.32	1SG 2
ATOM	2	CA	PRO	1	38.607	-21.369	7.975	1.00	33.32	1SG 3
ATOM	3	CD	PRO	1	39.877	-22.126	6.026	1.00	33.32	1SG 4
ATOM	4	CB	PRO	1	39.976	-21.869	8.434	1.00	33.32	1SG 5
ATOM	5	CG	PRO	1	40.848	-21.829	7.175	1.00	33.32	1SG 6
ATOM	6	C	PRO	1	38.437	-19.889	8.098	1.00	33.32	1SG 7
ATOM	7	O	PRO	1	38.643	-19.185	7.111	1.00	33.32	1SG 8
ATOM	8	N	MET	2	38.063	-19.396	9.296	1.00	34.27	1SG 9
ATOM	9	CA	MET	2	37.838	-17.993	9.495	1.00	34.27	1SG 10
ATOM	10	CB	MET	2	36.412	-17.676	9.998	1.00	34.27	1SG 11
ATOM	11	CG	MET	2	35.307	-17.862	8.948	1.00	34.27	1SG 12
ATOM	12	SD	MET	2	33.614	-17.595	9.564	1.00	34.27	1SG 13
ATOM	13	CE	MET	2	33.251	-19.346	9.888	1.00	34.27	1SG 14
ATOM	14	C	MET	2	38.803	-17.506	10.535	1.00	34.27	1SG 15
ATOM	15	O	MET	2	39.300	-18.279	11.355	1.00	34.27	1SG 16
ATOM	16	N	ALA	3	39.133	-16.199	10.477	1.00	38.92	1SG 17
ATOM	17	CA	ALA	3	39.983	-15.604	11.462	1.00	38.92	1SG 18
ATOM	18	CB	ALA	3	41.411	-15.329	10.948	1.00	38.92	1SG 19
ATOM	19	C	ALA	3	39.349	-14.301	11.837	1.00	38.92	1SG 20
ATOM	20	O	ALA	3	39.000	-13.487	10.980	1.00	38.92	1SG 21
ATOM	21	N	ARG	4	39.183	-14.074	13.150	1.00	46.12	1SG 22
ATOM	22	CA	ARG	4	38.537	-12.875	13.571	1.00	46.12	1SG 23

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	23	CB	ARG	4	37.249	-13.145	14.377	1.00	46.12	1SG 24
	ATOM	24	CG	ARG	4	36.232	-12.003	14.462	1.00	46.12	1SG 25
5	ATOM	25	CD	ARG	4	34.842	-12.538	14.822	1.00	46.12	1SG 26
	ATOM	26	NE	ARG	4	33.872	-11.424	14.644	1.00	46.12	1SG 27
	ATOM	27	CZ	ARG	4	33.274	-11.204	13.435	1.00	46.12	1SG 28
	ATOM	28	NH1	ARG	4	33.483	-12.053	12.386	1.00	46.12	1SG 29
	ATOM	29	NH2	ARG	4	32.496	-10.096	13.274	1.00	46.12	1SG 30
10	ATOM	30	C	ARG	4	39.502	-12.145	14.430	1.00	46.12	1SG 31
	ATOM	31	O	ARG	4	40.306	-12.725	15.161	1.00	46.12	1SG 32
	ATOM	32	N	TYR	5	39.434	-10.817	14.300	1.00	53.72	1SG 33
	ATOM	33	CA	TYR	5	40.226	-9.892	15.025	1.00	53.72	1SG 34
	ATOM	34	CB	TYR	5	40.325	-8.571	14.253	1.00	53.72	1SG 35
15	ATOM	35	CG	TYR	5	41.299	-8.710	13.136	1.00	53.72	1SG 36
	ATOM	36	CD1	TYR	5	41.167	-9.623	12.109	1.00	53.72	1SG 37
	ATOM	37	CD2	TYR	5	42.352	-7.834	13.127	1.00	53.72	1SG 38
	ATOM	38	CE1	TYR	5	42.123	-9.671	11.114	1.00	53.72	1SG 39
	ATOM	39	CE2	TYR	5	43.300	-7.878	12.141	1.00	53.72	1SG 40
20	ATOM	40	CZ	TYR	5	43.193	-8.801	11.135	1.00	53.72	1SG 41
	ATOM	41	OH	TYR	5	44.186	-8.828	10.133	1.00	53.72	1SG 42
	ATOM	42	C	TYR	5	39.479	-9.608	16.283	1.00	53.72	1SG 43
	ATOM	43	O	TYR	5	38.330	-9.165	16.257	1.00	53.72	1SG 44
	ATOM	44	N	TYR	6	40.096	-9.911	17.429	1.00	62.65	1SG 45
25	ATOM	45	CA	TYR	6	39.472	-9.583	18.671	1.00	62.65	1SG 46
	ATOM	46	CB	TYR	6	38.524	-10.643	19.262	1.00	62.65	1SG 47
	ATOM	47	CG	TYR	6	37.110	-10.362	18.901	1.00	62.65	1SG 48
	ATOM	48	CD1	TYR	6	36.660	-9.063	18.931	1.00	62.65	1SG 49
	ATOM	49	CD2	TYR	6	36.253	-11.367	18.519	1.00	62.65	1SG 50
30	ATOM	50	CE1	TYR	6	35.357	-8.767	18.627	1.00	62.65	1SG 51
	ATOM	51	CE2	TYR	6	34.943	-11.080	18.218	1.00	62.65	1SG 52
	ATOM	52	CZ	TYR	6	34.500	-9.779	18.276	1.00	62.65	1SG 53

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	53	OH	TYR	6	33.158	-9.470	17.976	1.00	62.65	1SG 54
	ATOM	54	C	TYR	6	40.493	-9.401	19.724	1.00	62.65	1SG 55
5	ATOM	55	O	TYR	6	41.618	-9.893	19.631	1.00	62.65	1SG 56
	ATOM	56	N	ILE	7	40.112	-8.638	20.761	1.00	68.54	1SG 57
	ATOM	57	CA	ILE	7	40.917	-8.675	21.927	1.00	68.54	1SG 58
	ATOM	58	CB	ILE	7	41.687	-7.463	22.346	1.00	68.54	1SG 59
	ATOM	59	CG2	ILE	7	40.686	-6.405	22.837	1.00	68.54	1SG 60
10	ATOM	60	CG1	ILE	7	42.697	-7.932	23.422	1.00	68.54	1SG 61
	ATOM	61	CD1	ILE	7	43.860	-6.998	23.705	1.00	68.54	1SG 62
	ATOM	62	C	ILE	7	39.977	-8.978	23.027	1.00	68.54	1SG 63
	ATOM	63	O	ILE	7	38.778	-8.712	22.936	1.00	68.54	1SG 64
	ATOM	64	N	ILE	8	40.522	-9.596	24.081	1.00	69.27	1SG 65
15	ATOM	65	CA	ILE	8	39.740	-9.984	25.199	1.00	69.27	1SG 66
	ATOM	66	CB	ILE	8	39.960	-11.419	25.578	1.00	69.27	1SG 67
	ATOM	67	CG2	ILE	8	39.168	-11.695	26.864	1.00	69.27	1SG 68
	ATOM	68	CG1	ILE	8	39.602	-12.344	24.403	1.00	69.27	1SG 69
	ATOM	69	CD1	ILE	8	38.170	-12.170	23.903	1.00	69.27	1SG 70
20	ATOM	70	C	ILE	8	40.221	-9.188	26.358	1.00	69.27	1SG 71
	ATOM	71	O	ILE	8	41.422	-8.990	26.537	1.00	69.27	1SG 72
	ATOM	72	N	LYS	9	39.268	-8.669	27.146	1.00	68.48	1SG 73
	ATOM	73	CA	LYS	9	39.604	-7.996	28.362	1.00	68.48	1SG 74
	ATOM	74	CB	LYS	9	39.034	-6.572	28.477	1.00	68.48	1SG 75
25	ATOM	75	CG	LYS	9	39.763	-5.543	27.617	1.00	68.48	1SG 76
	ATOM	76	CD	LYS	9	38.943	-4.279	27.355	1.00	68.48	1SG 77
	ATOM	77	CE	LYS	9	39.657	-2.997	27.770	1.00	68.48	1SG 78
	ATOM	78	NZ	LYS	9	40.955	-2.903	27.070	1.00	68.48	1SG 79
	ATOM	79	C	LYS	9	38.959	-8.817	29.422	1.00	68.48	1SG 80
30	ATOM	80	O	LYS	9	37.956	-9.482	29.162	1.00	68.48	1SG 81
	ATOM	81	N	TYR	10	39.482	-8.796	30.660	1.00	64.11	1SG 82
	ATOM	82	CA	TYR	10	38.779	-9.629	31.582	1.00	64.11	1SG 83

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	83	CB	TYR	10	39.569	-10.540	32.567	1.00	64.11	1SG 84
ATOM	84	CG	TYR	10	40.443	-9.815	33.532	1.00	64.11	1SG 85
ATOM	85	CD1	TYR	10	39.929	-8.879	34.391	1.00	64.11	1SG 86
ATOM	86	CD2	TYR	10	41.775	-10.140	33.638	1.00	64.11	1SG 87
ATOM	87	CE1	TYR	10	40.739	-8.227	35.289	1.00	64.11	1SG 88
ATOM	88	CE2	TYR	10	42.596	-9.496	34.531	1.00	64.11	1SG 89
ATOM	89	CZ	TYR	10	42.077	-8.530	35.355	1.00	64.11	1SG 90
ATOM	90	OH	TYR	10	42.911	-7.862	36.276	1.00	64.11	1SG 91
ATOM	91	C	TYR	10	37.811	-8.756	32.295	1.00	64.11	1SG 92
ATOM	92	O	TYR	10	37.697	-7.572	31.989	1.00	64.11	1SG 93
ATOM	93	N	ALA	11	37.059	-9.328	33.245	1.00	60.60	1SG 94
ATOM	94	CA	ALA	11	35.961	-8.637	33.852	1.00	60.60	1SG 95
ATOM	95	CB	ALA	11	35.310	-9.425	34.997	1.00	60.60	1SG 96
ATOM	96	C	ALA	11	36.426	-7.331	34.410	1.00	60.60	1SG 97
ATOM	97	O	ALA	11	35.649	-6.381	34.476	1.00	60.60	1SG 98
ATOM	98	N	ASP	12	37.685	-7.253	34.872	1.00	58.92	1SG 99
ATOM	99	CA	ASP	12	38.131	-6.025	35.460	1.00	58.92	1SG 100
ATOM	100	CB	ASP	12	39.042	-6.249	36.673	1.00	58.92	1SG 101
ATOM	101	CG	ASP	12	38.099	-6.632	37.805	1.00	58.92	1SG 102
ATOM	102	OD1	ASP	12	36.965	-6.081	37.815	1.00	58.92	1SG 103
ATOM	103	OD2	ASP	12	38.481	-7.474	38.659	1.00	58.92	1SG 104
ATOM	104	C	ASP	12	38.841	-5.144	34.473	1.00	58.92	1SG 105
ATOM	105	O	ASP	12	39.777	-4.431	34.833	1.00	58.92	1SG 106
ATOM	106	N	GLN	13	38.384	-5.143	33.207	1.00	57.45	1SG 107
ATOM	107	CA	GLN	13	38.868	-4.238	32.199	1.00	57.45	1SG 108
ATOM	108	CB	GLN	13	38.526	-2.774	32.511	1.00	57.45	1SG 109
ATOM	109	CG	GLN	13	37.023	-2.500	32.520	1.00	57.45	1SG 110
ATOM	110	CD	GLN	13	36.544	-2.555	31.077	1.00	57.45	1SG 111
ATOM	111	OE1	GLN	13	37.229	-2.084	30.170	1.00	57.45	1SG 112
ATOM	112	NE2	GLN	13	35.343	-3.150	30.850	1.00	57.45	1SG 113

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	113	C	GLN	13	40.349	-4.332	32.019	1.00	57.45	1SG 114
	ATOM	114	O	GLN	13	41.004	-3.343	31.696	1.00	57.45	1SG 115
5	ATOM	115	N	LYS	14	40.910	-5.533	32.207	1.00	58.16	1SG 116
	ATOM	116	CA	LYS	14	42.302	-5.776	31.993	1.00	58.16	1SG 117
	ATOM	117	CB	LYS	14	42.758	-6.983	32.837	1.00	58.16	1SG 118
	ATOM	118	CG	LYS	14	44.072	-7.653	32.443	1.00	58.16	1SG 119
	ATOM	119	CD	LYS	14	43.997	-8.498	31.161	1.00	58.16	1SG 120
10	ATOM	120	CE	LYS	14	42.643	-9.198	31.004	1.00	58.16	1SG 121
	ATOM	121	NZ	LYS	14	42.479	-9.776	29.655	1.00	58.16	1SG 122
	ATOM	122	C	LYS	14	42.470	-6.088	30.538	1.00	58.16	1SG 123
	ATOM	123	O	LYS	14	41.623	-6.748	29.942	1.00	58.16	1SG 124
	ATOM	124	N	ALA	15	43.595	-5.636	29.944	1.00	54.24	1SG 125
15	ATOM	125	CA	ALA	15	43.937	-5.866	28.571	1.00	54.24	1SG 126
	ATOM	126	CB	ALA	15	44.398	-4.595	27.835	1.00	54.24	1SG 127
	ATOM	127	C	ALA	15	45.092	-6.822	28.577	1.00	54.24	1SG 128
	ATOM	128	O	ALA	15	45.767	-6.973	29.593	1.00	54.24	1SG 129
	ATOM	129	N	LEU	16	45.325	-7.528	27.450	1.00	50.90	1SG 130
20	ATOM	130	CA	LEU	16	46.381	-8.503	27.392	1.00	50.90	1SG 131
	ATOM	131	CB	LEU	16	45.934	-9.852	26.801	1.00	50.90	1SG 132
	ATOM	132	CG	LEU	16	44.833	-10.544	27.623	1.00	50.90	1SG 133
	ATOM	133	CD2	LEU	16	45.216	-10.618	29.105	1.00	50.90	1SG 134
	ATOM	134	CD1	LEU	16	44.458	-11.913	27.036	1.00	50.90	1SG 135
25	ATOM	135	C	LEU	16	47.460	-7.999	26.483	1.00	50.90	1SG 136
	ATOM	136	O	LEU	16	47.176	-7.396	25.449	1.00	50.90	1SG 137
	ATOM	137	N	TYR	17	48.737	-8.228	26.863	1.00	48.25	1SG 138
	ATOM	138	CA	TYR	17	49.853	-7.817	26.056	1.00	48.25	1SG 139
	ATOM	139	CB	TYR	17	50.452	-6.469	26.510	1.00	48.25	1SG 140
30	ATOM	140	CG	TYR	17	51.599	-6.103	25.630	1.00	48.25	1SG 141
	ATOM	141	CD1	TYR	17	51.380	-5.535	24.396	1.00	48.25	1SG 142
	ATOM	142	CD2	TYR	17	52.893	-6.346	26.027	1.00	48.25	1SG 143

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	143	CE1	TYR	17	52.432	-5.197	23.577	1.00	48.25	1SG 144
ATOM	144	CE2	TYR	17	53.949	-6.008	25.213	1.00	48.25	1SG 145
ATOM	145	CZ	TYR	17	53.722	-5.432	23.988	1.00	48.25	1SG 146
ATOM	146	OH	TYR	17	54.810	-5.089	23.158	1.00	48.25	1SG 147
ATOM	147	C	TYR	17	50.902	-8.889	26.148	1.00	48.25	1SG 148
ATOM	148	O	TYR	17	50.844	-9.745	27.030	1.00	48.25	1SG 149
ATOM	149	N	THR	18	51.884	-8.878	25.221	1.00	46.19	1SG 150
ATOM	150	CA	THR	18	52.927	-9.867	25.193	1.00	46.19	1SG 151
ATOM	151	CB	THR	18	53.441	-10.097	23.801	1.00	46.19	1SG 152
ATOM	152	OG1	THR	18	52.393	-10.552	22.961	1.00	46.19	1SG 153
ATOM	153	CG2	THR	18	54.575	-11.125	23.838	1.00	46.19	1SG 154
ATOM	154	C	THR	18	54.072	-9.347	26.002	1.00	46.19	1SG 155
ATOM	155	O	THR	18	54.883	-8.563	25.513	1.00	46.19	1SG 156
ATOM	156	N	ARG	19	54.171	-9.775	27.276	1.00	52.23	1SG 157
ATOM	157	CA	ARG	19	55.219	-9.263	28.109	1.00	52.23	1SG 158
ATOM	158	CB	ARG	19	55.131	-9.774	29.555	1.00	52.23	1SG 159
ATOM	159	CG	ARG	19	56.226	-9.206	30.459	1.00	52.23	1SG 160
ATOM	160	CD	ARG	19	56.158	-9.710	31.902	1.00	52.23	1SG 161
ATOM	161	NE	ARG	19	57.280	-9.070	32.643	1.00	52.23	1SG 162
ATOM	162	CZ	ARG	19	58.522	-9.636	32.630	1.00	52.23	1SG 163
ATOM	163	NH1	ARG	19	58.738	-10.798	31.947	1.00	52.23	1SG 164
ATOM	164	NH2	ARG	19	59.551	-9.035	33.296	1.00	52.23	1SG 165
ATOM	165	C	ARG	19	56.546	-9.680	27.566	1.00	52.23	1SG 166
ATOM	166	O	ARG	19	57.407	-8.846	27.287	1.00	52.23	1SG 167
ATOM	167	N	ASP	20	56.735	-11.000	27.381	1.00	59.27	1SG 168
ATOM	168	CA	ASP	20	57.964	-11.494	26.838	1.00	59.27	1SG 169
ATOM	169	CB	ASP	20	58.963	-11.966	27.907	1.00	59.27	1SG 170
ATOM	170	CG	ASP	20	60.296	-12.237	27.221	1.00	59.27	1SG 171
ATOM	171	OD1	ASP	20	60.343	-12.176	25.963	1.00	59.27	1SG 172
ATOM	172	OD2	ASP	20	61.289	-12.500	27.950	1.00	59.27	1SG 173

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	173 C	ASP	20	57.571	-12.683	26.042	1.00	59.27	1SG 174
	ATOM	174 O	ASP	20	58.291	-13.675	25.961	1.00	59.27	1SG 175
	ATOM	175 N	GLY	21	56.385	-12.591	25.427	1.00	65.03	1SG 176
	ATOM	176 CA	GLY	21	55.889	-13.646	24.610	1.00	65.03	1SG 177
	ATOM	177 C	GLY	21	54.895	-14.444	25.404	1.00	65.03	1SG 178
	ATOM	178 O	GLY	21	54.020	-15.079	24.818	1.00	65.03	1SG 179
	ATOM	179 N	GLN	22	55.002	-14.432	26.754	1.00	68.58	1SG 180
	ATOM	180 CA	GLN	22	54.145	-15.243	27.581	1.00	68.58	1SG 181
	ATOM	181 CB	GLN	22	54.580	-15.294	29.060	1.00	68.58	1SG 182
	ATOM	182 CG	GLN	22	54.492	-13.962	29.809	1.00	68.58	1SG 183
	ATOM	183 CD	GLN	22	55.821	-13.234	29.674	1.00	68.58	1SG 184
	ATOM	184 OE1	GLN	22	56.058	-12.234	30.349	1.00	68.58	1SG 185
	ATOM	185 NE2	GLN	22	56.717	-13.751	28.790	1.00	68.58	1SG 186
	ATOM	186 C	GLN	22	52.705	-14.815	27.559	1.00	68.58	1SG 187
	ATOM	187 O	GLN	22	51.859	-15.630	27.215	1.00	68.58	1SG 188
	ATOM	188 N	LEU	23	52.401	-13.539	27.894	1.00	63.11	1SG 189
	ATOM	189 CA	LEU	23	51.081	-12.938	27.965	1.00	63.11	1SG 190
	ATOM	190 CB	LEU	23	49.859	-13.873	27.803	1.00	63.11	1SG 191
	ATOM	191 CG	LEU	23	49.580	-14.387	26.374	1.00	63.11	1SG 192
	ATOM	192 CD2	LEU	23	49.467	-13.231	25.371	1.00	63.11	1SG 193
	ATOM	193 CD1	LEU	23	48.345	-15.301	26.346	1.00	63.11	1SG 194
	ATOM	194 C	LEU	23	50.939	-12.331	29.330	1.00	63.11	1SG 195
	ATOM	195 O	LEU	23	51.090	-13.017	30.341	1.00	63.11	1SG 196
	ATOM	196 N	LEU	24	50.622	-11.022	29.386	1.00	55.20	1SG 197
	ATOM	197 CA	LEU	24	50.498	-10.321	30.632	1.00	55.20	1SG 198
	ATOM	198 CB	LEU	24	51.666	-9.321	30.820	1.00	55.20	1SG 199
	ATOM	199 CG	LEU	24	51.703	-8.467	32.108	1.00	55.20	1SG 200
	ATOM	200 CD2	LEU	24	50.544	-7.460	32.181	1.00	55.20	1SG 201
	ATOM	201 CD1	LEU	24	53.044	-7.724	32.211	1.00	55.20	1SG 202
	ATOM	202 C	LEU	24	49.194	-9.586	30.619	1.00	55.20	1SG 203

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	203	O	LEU	24	48.705	-9.187	29.564	1.00	55.20	1SG 204
	ATOM	204	N	VAL	25	48.578	-9.430	31.811	1.00	49.24	1SG 205
5	ATOM	205	CA	VAL	25	47.336	-8.727	31.945	1.00	49.24	1SG 206
	ATOM	206	CB	VAL	25	46.321	-9.527	32.715	1.00	49.24	1SG 207
	ATOM	207	CG1	VAL	25	46.013	-10.808	31.930	1.00	49.24	1SG 208
	ATOM	208	CG2	VAL	25	46.856	-9.811	34.130	1.00	49.24	1SG 209
	ATOM	209	C	VAL	25	47.609	-7.472	32.725	1.00	49.24	1SG 210
10	ATOM	210	O	VAL	25	48.274	-7.498	33.760	1.00	49.24	1SG 211
	ATOM	211	N	GLY	26	47.094	-6.326	32.233	1.00	45.56	1SG 212
	ATOM	212	CA	GLY	26	47.263	-5.059	32.890	1.00	45.56	1SG 213
	ATOM	213	C	GLY	26	46.128	-4.210	32.431	1.00	45.56	1SG 214
	ATOM	214	O	GLY	26	45.360	-4.638	31.579	1.00	45.56	1SG 215
15	ATOM	215	N	ASP	27	45.967	-2.990	32.979	1.00	47.30	1SG 216
	ATOM	216	CA	ASP	27	44.862	-2.193	32.521	1.00	47.30	1SG 217
	ATOM	217	CB	ASP	27	44.021	-1.595	33.663	1.00	47.30	1SG 218
	ATOM	218	CG	ASP	27	42.744	-1.012	33.068	1.00	47.30	1SG 219
	ATOM	219	OD1	ASP	27	42.587	-1.075	31.819	1.00	47.30	1SG 220
20	ATOM	220	OD2	ASP	27	41.905	-0.502	33.857	1.00	47.30	1SG 221
	ATOM	221	C	ASP	27	45.405	-1.055	31.712	1.00	47.30	1SG 222
	ATOM	222	O	ASP	27	45.828	-0.046	32.270	1.00	47.30	1SG 223
	ATOM	223	N	PRO	28	45.452	-1.210	30.412	1.00	51.05	1SG 224
	ATOM	224	CA	PRO	28	45.924	-0.125	29.594	1.00	51.05	1SG 225
25	ATOM	225	CD	PRO	28	45.902	-2.484	29.878	1.00	51.05	1SG 226
	ATOM	226	CB	PRO	28	46.569	-0.751	28.358	1.00	51.05	1SG 227
	ATOM	227	CG	PRO	28	46.948	-2.163	28.807	1.00	51.05	1SG 228
	ATOM	228	C	PRO	28	44.837	0.820	29.200	1.00	51.05	1SG 229
	ATOM	229	O	PRO	28	43.665	0.453	29.259	1.00	51.05	1SG 230
30	ATOM	230	N	VAL	29	45.222	2.046	28.799	1.00	52.42	1SG 231
	ATOM	231	CA	VAL	29	44.305	3.008	28.264	1.00	52.42	1SG 232
	ATOM	232	CB	VAL	29	43.622	3.844	29.307	1.00	52.42	1SG 233

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	233	CG1	VAL	29	44.688	4.667	30.050	1.00	52.42	1SG 234
	ATOM	234	CG2	VAL	29	42.550	4.702	28.615	1.00	52.42	1SG 235
5	ATOM	235	C	VAL	29	45.145	3.931	27.445	1.00	52.42	1SG 236
	ATOM	236	O	VAL	29	46.307	4.136	27.788	1.00	52.42	1SG 237
	ATOM	237	N	ALA	30	44.595	4.464	26.328	1.00	50.57	1SG 238
	ATOM	238	CA	ALA	30	45.268	5.424	25.487	1.00	50.57	1SG 239
	ATOM	239	CB	ALA	30	46.354	6.299	26.148	1.00	50.57	1SG 240
10	ATOM	240	C	ALA	30	45.854	4.749	24.290	1.00	50.57	1SG 241
	ATOM	241	O	ALA	30	45.433	3.667	23.884	1.00	50.57	1SG 242
	ATOM	242	N	ASP	31	46.852	5.417	23.686	1.00	46.54	1SG 243
	ATOM	243	CA	ASP	31	47.521	4.975	22.498	1.00	46.54	1SG 244
	ATOM	244	CB	ASP	31	48.617	5.960	22.057	1.00	46.54	1SG 245
15	ATOM	245	CG	ASP	31	49.084	5.578	20.661	1.00	46.54	1SG 246
	ATOM	246	OD1	ASP	31	48.539	4.591	20.100	1.00	46.54	1SG 247
	ATOM	247	OD2	ASP	31	49.995	6.274	20.138	1.00	46.54	1SG 248
	ATOM	248	C	ASP	31	48.175	3.660	22.788	1.00	46.54	1SG 249
	ATOM	249	O	ASP	31	48.309	2.815	21.905	1.00	46.54	1SG 250
20	ATOM	250	N	ASN	32	48.583	3.454	24.053	1.00	40.79	1SG 251
	ATOM	251	CA	ASN	32	49.270	2.266	24.471	1.00	40.79	1SG 252
	ATOM	252	CB	ASN	32	49.772	2.310	25.928	1.00	40.79	1SG 253
	ATOM	253	CG	ASN	32	48.640	2.628	26.892	1.00	40.79	1SG 254
	ATOM	254	OD1	ASN	32	48.774	3.576	27.664	1.00	40.79	1SG 255
25	ATOM	255	ND2	ASN	32	47.530	1.842	26.871	1.00	40.79	1SG 256
	ATOM	256	C	ASN	32	48.397	1.069	24.255	1.00	40.79	1SG 257
	ATOM	257	O	ASN	32	48.883	-0.059	24.195	1.00	40.79	1SG 258
	ATOM	258	N	CYS	33	47.080	1.293	24.122	1.00	37.36	1SG 259
	ATOM	259	CA	CYS	33	46.117	0.245	23.940	1.00	37.36	1SG 260
30	ATOM	260	CB	CYS	33	44.689	0.778	23.733	1.00	37.36	1SG 261
	ATOM	261	SG	CYS	33	44.007	1.554	25.229	1.00	37.36	1SG 262
	ATOM	262	C	CYS	33	46.486	-0.530	22.710	1.00	37.36	1SG 263

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	263	O	CYS	33	46.129	-1.699	22.572	1.00	37.36	1SG 264
ATOM	264	N	CYS	34	47.230	0.099	21.787	1.00	40.37	1SG 265
ATOM	265	CA	CYS	34	47.592	-0.501	20.532	1.00	40.37	1SG 266
ATOM	266	CB	CYS	34	48.510	0.400	19.692	1.00	40.37	1SG 267
ATOM	267	SG	CYS	34	47.702	1.958	19.226	1.00	40.37	1SG 268
ATOM	268	C	CYS	34	48.342	-1.777	20.780	1.00	40.37	1SG 269
ATOM	269	O	CYS	34	48.269	-2.708	19.978	1.00	40.37	1SG 270
ATOM	270	N	ALA	35	49.070	-1.860	21.906	1.00	48.30	1SG 271
ATOM	271	CA	ALA	35	49.904	-2.985	22.234	1.00	48.30	1SG 272
ATOM	272	CB	ALA	35	50.597	-2.812	23.595	1.00	48.30	1SG 273
ATOM	273	C	ALA	35	49.073	-4.241	22.276	1.00	48.30	1SG 274
ATOM	274	O	ALA	35	49.558	-5.330	21.976	1.00	48.30	1SG 275
ATOM	275	N	GLU	36	47.797	-4.116	22.674	1.00	58.23	1SG 276
ATOM	276	CA	GLU	36	46.849	-5.194	22.803	1.00	58.23	1SG 277
ATOM	277	CB	GLU	36	45.453	-4.623	23.095	1.00	58.23	1SG 278
ATOM	278	CG	GLU	36	45.311	-3.973	24.473	1.00	58.23	1SG 279
ATOM	279	CD	GLU	36	43.946	-3.301	24.528	1.00	58.23	1SG 280
ATOM	280	OE1	GLU	36	43.488	-2.810	23.459	1.00	58.23	1SG 281
ATOM	281	OE2	GLU	36	43.341	-3.267	25.631	1.00	58.23	1SG 282
ATOM	282	C	GLU	36	46.750	-5.977	21.506	1.00	58.23	1SG 283
ATOM	283	O	GLU	36	46.914	-5.403	20.430	1.00	58.23	1SG 284
ATOM	284	N	LYS	37	46.514	-7.324	21.570	1.00	65.72	1SG 285
ATOM	285	CA	LYS	37	46.371	-8.106	20.348	1.00	65.72	1SG 286
ATOM	286	CB	LYS	37	47.679	-8.177	19.532	1.00	65.72	1SG 287
ATOM	287	CG	LYS	37	48.888	-8.600	20.360	1.00	65.72	1SG 288
ATOM	288	CD	LYS	37	48.773	-10.032	20.869	1.00	65.72	1SG 289
ATOM	289	CE	LYS	37	49.819	-10.374	21.924	1.00	65.72	1SG 290
ATOM	290	NZ	LYS	37	49.625	-11.761	22.399	1.00	65.72	1SG 291
ATOM	291	C	LYS	37	45.835	-9.526	20.583	1.00	65.72	1SG 292
ATOM	292	O	LYS	37	45.594	-9.896	21.732	1.00	65.72	1SG 293

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	293	N	ILE	38	45.553	-10.301	19.469	1.00	67.02	1SG 294
ATOM	294	CA	ILE	38	45.168	-11.718	19.359	1.00	67.02	1SG 295
ATOM	295	CB	ILE	38	44.552	-12.414	20.547	1.00	67.02	1SG 296
ATOM	296	CG2	ILE	38	45.618	-12.626	21.637	1.00	67.02	1SG 297
ATOM	297	CG1	ILE	38	43.245	-11.736	20.969	1.00	67.02	1SG 298
ATOM	298	CD1	ILE	38	42.388	-12.628	21.865	1.00	67.02	1SG 299
ATOM	299	C	ILE	38	44.222	-11.975	18.205	1.00	67.02	1SG 300
ATOM	300	O	ILE	38	43.388	-11.135	17.873	1.00	67.02	1SG 301
ATOM	301	N	CYS	39	44.369	-13.149	17.528	1.00	63.20	1SG 302
ATOM	302	CA	CYS	39	43.484	-13.568	16.459	1.00	63.20	1SG 303
ATOM	303	CB	CYS	39	44.215	-13.958	15.161	1.00	63.20	1SG 304
ATOM	304	SG	CYS	39	45.292	-15.406	15.378	1.00	63.20	1SG 305
ATOM	305	C	CYS	39	42.743	-14.805	16.914	1.00	63.20	1SG 306
ATOM	306	O	CYS	39	43.334	-15.698	17.519	1.00	63.20	1SG 307
ATOM	307	N	ILE	40	41.420	-14.887	16.619	1.00	57.92	1SG 308
ATOM	308	CA	ILE	40	40.582	-15.994	17.020	1.00	57.92	1SG 309
ATOM	309	CB	ILE	40	39.397	-15.569	17.847	1.00	57.92	1SG 310
ATOM	310	CG2	ILE	40	38.402	-16.736	17.900	1.00	57.92	1SG 311
ATOM	311	CG1	ILE	40	39.845	-15.055	19.228	1.00	57.92	1SG 312
ATOM	312	CD1	ILE	40	38.738	-14.341	20.005	1.00	57.92	1SG 313
ATOM	313	C	ILE	40	40.056	-16.676	15.791	1.00	57.92	1SG 314
ATOM	314	O	ILE	40	39.465	-16.043	14.916	1.00	57.92	1SG 315
ATOM	315	N	LEU	41	40.257	-18.009	15.707	1.00	55.12	1SG 316
ATOM	316	CA	LEU	41	39.876	-18.774	14.552	1.00	55.12	1SG 317
ATOM	317	CB	LEU	41	41.103	-19.537	13.995	1.00	55.12	1SG 318
ATOM	318	CG	LEU	41	40.948	-20.324	12.676	1.00	55.12	1SG 319
ATOM	319	CD2	LEU	41	39.844	-21.381	12.744	1.00	55.12	1SG 320
ATOM	320	CD1	LEU	41	42.282	-20.976	12.280	1.00	55.12	1SG 321
ATOM	321	C	LEU	41	38.821	-19.751	14.973	1.00	55.12	1SG 322
ATOM	322	O	LEU	41	38.992	-20.498	15.933	1.00	55.12	1SG 323

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	323 N	PRO	42	37.697	-19.717	14.308	1.00	50.96	1SG 324
	ATOM	324 CA	PRO	42	36.686	-20.695	14.606	1.00	50.96	1SG 325
5	ATOM	325 CD	PRO	42	37.122	-18.442	13.914	1.00	50.96	1SG 326
	ATOM	326 CB	PRO	42	35.378	-20.132	14.058	1.00	50.96	1SG 327
	ATOM	327 CG	PRO	42	35.605	-18.608	14.090	1.00	50.96	1SG 328
	ATOM	328 C	PRO	42	37.113	-22.001	14.024	1.00	50.96	1SG 329
	ATOM	329 O	PRO	42	37.212	-22.097	12.802	1.00	50.96	1SG 330
10	ATOM	330 N	ASN	43	37.272	-23.044	14.857	1.00	45.74	1SG 331
	ATOM	331 CA	ASN	43	37.798	-24.277	14.354	1.00	45.74	1SG 332
	ATOM	332 CB	ASN	43	39.057	-24.748	15.102	1.00	45.74	1SG 333
	ATOM	333 CG	ASN	43	38.669	-25.002	16.552	1.00	45.74	1SG 334
	ATOM	334 OD1	ASN	43	38.046	-24.157	17.193	1.00	45.74	1SG 335
15	ATOM	335 ND2	ASN	43	39.040	-26.197	17.085	1.00	45.74	1SG 336
	ATOM	336 C	ASN	43	36.768	-25.335	14.520	1.00	45.74	1SG 337
	ATOM	337 O	ASN	43	35.690	-25.107	15.065	1.00	45.74	1SG 338
	ATOM	338 N	ARG	44	37.078	-26.529	13.986	1.00	40.33	1SG 339
	ATOM	339 CA	ARG	44	36.185	-27.637	14.099	1.00	40.33	1SG 340
20	ATOM	340 CB	ARG	44	36.409	-28.706	13.014	1.00	40.33	1SG 341
	ATOM	341 CG	ARG	44	37.827	-29.279	12.979	1.00	40.33	1SG 342
	ATOM	342 CD	ARG	44	38.132	-30.232	14.133	1.00	40.33	1SG 343
	ATOM	343 NE	ARG	44	37.286	-31.441	13.935	1.00	40.33	1SG 344
	ATOM	344 CZ	ARG	44	37.213	-32.380	14.921	1.00	40.33	1SG 345
25	ATOM	345 NH1	ARG	44	37.931	-32.213	16.070	1.00	40.33	1SG 346
	ATOM	346 NH2	ARG	44	36.421	-33.480	14.760	1.00	40.33	1SG 347
	ATOM	347 C	ARG	44	36.368	-28.241	15.453	1.00	40.33	1SG 348
	ATOM	348 O	ARG	44	37.446	-28.182	16.042	1.00	40.33	1SG 349
	ATOM	349 N	GLY	45	35.282	-28.823	15.992	1.00	32.71	1SG 350
30	ATOM	350 CA	GLY	45	35.330	-29.414	17.294	1.00	32.71	1SG 351
	ATOM	351 C	GLY	45	33.922	-29.725	17.663	1.00	32.71	1SG 352
	ATOM	352 O	GLY	45	33.034	-29.730	16.812	1.00	32.71	1SG 353

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	353	N	LEU	46	33.680	-29.994	18.958	1.00	30.21	1SG 354
	ATOM	354	CA	LEU	46	32.346	-30.312	19.365	1.00	30.21	1SG 355
	ATOM	355	CB	LEU	46	32.285	-31.110	20.678	1.00	30.21	1SG 356
	ATOM	356	CG	LEU	46	32.992	-32.477	20.599	1.00	30.21	1SG 357
	ATOM	357	CD2	LEU	46	32.496	-33.297	19.398	1.00	30.21	1SG 358
	ATOM	358	CD1	LEU	46	32.888	-33.238	21.930	1.00	30.21	1SG 359
	ATOM	359	C	LEU	46	31.642	-29.018	19.593	1.00	30.21	1SG 360
	ATOM	360	O	LEU	46	32.084	-28.186	20.385	1.00	30.21	1SG 361
	ATOM	361	N	ASP	47	30.522	-28.813	18.875	1.00	32.57	1SG 362
	ATOM	362	CA	ASP	47	29.774	-27.605	19.029	1.00	32.57	1SG 363
	ATOM	363	CB	ASP	47	29.361	-26.958	17.694	1.00	32.57	1SG 364
	ATOM	364	CG	ASP	47	28.692	-25.621	17.988	1.00	32.57	1SG 365
	ATOM	365	OD1	ASP	47	28.576	-25.263	19.190	1.00	32.57	1SG 366
	ATOM	366	OD2	ASP	47	28.292	-24.935	17.009	1.00	32.57	1SG 367
	ATOM	367	C	ASP	47	28.518	-27.948	19.755	1.00	32.57	1SG 368
	ATOM	368	O	ASP	47	27.733	-28.790	19.322	1.00	32.57	1SG 369
	ATOM	369	N	ARG	48	28.324	-27.293	20.909	1.00	34.94	1SG 370
	ATOM	370	CA	ARG	48	27.162	-27.439	21.728	1.00	34.94	1SG 371
	ATOM	371	CB	ARG	48	27.404	-28.219	23.033	1.00	34.94	1SG 372
	ATOM	372	CG	ARG	48	27.668	-29.712	22.828	1.00	34.94	1SG 373
	ATOM	373	CD	ARG	48	27.895	-30.472	24.137	1.00	34.94	1SG 374
	ATOM	374	NE	ARG	48	28.133	-31.902	23.795	1.00	34.94	1SG 375
	ATOM	375	CZ	ARG	48	27.092	-32.785	23.791	1.00	34.94	1SG 376
	ATOM	376	NH1	ARG	48	25.837	-32.357	24.116	1.00	34.94	1SG 377
	ATOM	377	NH2	ARG	48	27.305	-34.094	23.468	1.00	34.94	1SG 378
	ATOM	378	C	ARG	48	26.868	-26.041	22.115	1.00	34.94	1SG 379
	ATOM	379	O	ARG	48	27.113	-25.121	21.336	1.00	34.94	1SG 380
	ATOM	380	N	THR	49	26.299	-25.829	23.311	1.00	39.86	1SG 381
	ATOM	381	CA	THR	49	26.166	-24.458	23.674	1.00	39.86	1SG 382
	ATOM	382	CB	THR	49	25.210	-24.227	24.807	1.00	39.86	1SG 383

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	383	OG1	THR	49	23.915	-24.694	24.459	1.00	39.86	1SG 384
	ATOM	384	CG2	THR	49	25.167	-22.721	25.119	1.00	39.86	1SG 385
	ATOM	385	C	THR	49	27.533	-24.075	24.149	1.00	39.86	1SG 386
	ATOM	386	O	THR	49	27.793	-24.020	25.350	1.00	39.86	1SG 387
	ATOM	387	N	LYS	50	28.446	-23.834	23.181	1.00	43.04	1SG 388
	ATOM	388	CA	LYS	50	29.815	-23.473	23.400	1.00	43.04	1SG 389
	ATOM	389	CB	LYS	50	30.522	-24.412	24.391	1.00	43.04	1SG 390
	ATOM	390	CG	LYS	50	31.765	-23.807	25.041	1.00	43.04	1SG 391
	ATOM	391	CD	LYS	50	32.193	-24.554	26.307	1.00	43.04	1SG 392
	ATOM	392	CE	LYS	50	33.254	-23.820	27.128	1.00	43.04	1SG 393
	ATOM	393	NZ	LYS	50	33.489	-24.536	28.402	1.00	43.04	1SG 394
	ATOM	394	C	LYS	50	30.457	-23.608	22.050	1.00	43.04	1SG 395
	ATOM	395	O	LYS	50	30.036	-24.446	21.253	1.00	43.04	1SG 396
	ATOM	396	N	VAL	51	31.478	-22.784	21.732	1.00	44.03	1SG 397
	ATOM	397	CA	VAL	51	32.054	-22.899	20.419	1.00	44.03	1SG 398
	ATOM	398	CB	VAL	51	31.843	-21.683	19.565	1.00	44.03	1SG 399
	ATOM	399	CG1	VAL	51	32.582	-21.888	18.231	1.00	44.03	1SG 400
	ATOM	400	CG2	VAL	51	30.332	-21.442	19.414	1.00	44.03	1SG 401
	ATOM	401	C	VAL	51	33.534	-23.080	20.542	1.00	44.03	1SG 402
	ATOM	402	O	VAL	51	34.192	-22.431	21.352	1.00	44.03	1SG 403
	ATOM	403	N	PRO	52	34.065	-23.976	19.749	1.00	46.94	1SG 404
	ATOM	404	CA	PRO	52	35.488	-24.189	19.757	1.00	46.94	1SG 405
	ATOM	405	CD	PRO	52	33.341	-25.203	19.455	1.00	46.94	1SG 406
	ATOM	406	CB	PRO	52	35.721	-25.552	19.110	1.00	46.94	1SG 407
	ATOM	407	CG	PRO	52	34.409	-26.308	19.385	1.00	46.94	1SG 408
	ATOM	408	C	PRO	52	36.201	-23.065	19.077	1.00	46.94	1SG 409
	ATOM	409	O	PRO	52	35.729	-22.589	18.044	1.00	46.94	1SG 410
	ATOM	410	N	ILE	53	37.355	-22.648	19.633	1.00	47.21	1SG 411
	ATOM	411	CA	ILE	53	38.096	-21.543	19.107	1.00	47.21	1SG 412
	ATOM	412	CB	ILE	53	37.817	-20.286	19.889	1.00	47.21	1SG 413

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	413	CG2	ILE	53	38.805	-19.184	19.483	1.00	47.21	1SG 414
	ATOM	414	CG1	ILE	53	36.340	-19.888	19.733	1.00	47.21	1SG 415
	ATOM	415	CD1	ILE	53	35.935	-19.585	18.290	1.00	47.21	1SG 416
	ATOM	416	C	ILE	53	39.554	-21.855	19.255	1.00	47.21	1SG 417
	ATOM	417	O	ILE	53	39.958	-22.644	20.108	1.00	47.21	1SG 418
	ATOM	418	N	PHE	54	40.371	-21.242	18.378	1.00	47.50	1SG 419
	ATOM	419	CA	PHE	54	41.799	-21.330	18.379	1.00	47.50	1SG 420
	ATOM	420	CB	PHE	54	42.309	-21.800	17.009	1.00	47.50	1SG 421
	ATOM	421	CG	PHE	54	43.788	-21.828	17.028	1.00	47.50	1SG 422
	ATOM	422	CD1	PHE	54	44.457	-22.916	17.533	1.00	47.50	1SG 423
	ATOM	423	CD2	PHE	54	44.494	-20.761	16.528	1.00	47.50	1SG 424
	ATOM	424	CE1	PHE	54	45.827	-22.933	17.542	1.00	47.50	1SG 425
	ATOM	425	CE2	PHE	54	45.865	-20.772	16.534	1.00	47.50	1SG 426
	ATOM	426	CZ	PHE	54	46.522	-21.863	17.043	1.00	47.50	1SG 427
	ATOM	427	C	PHE	54	42.258	-19.920	18.601	1.00	47.50	1SG 428
	ATOM	428	O	PHE	54	41.680	-18.988	18.045	1.00	47.50	1SG 429
	ATOM	429	N	LEU	55	43.290	-19.711	19.446	1.00	47.90	1SG 430
	ATOM	430	CA	LEU	55	43.731	-18.372	19.714	1.00	47.90	1SG 431
	ATOM	431	CB	LEU	55	43.708	-18.034	21.214	1.00	47.90	1SG 432
	ATOM	432	CG	LEU	55	42.301	-18.055	21.837	1.00	47.90	1SG 433
	ATOM	433	CD2	LEU	55	41.342	-17.140	21.061	1.00	47.90	1SG 434
	ATOM	434	CD1	LEU	55	42.349	-17.718	23.335	1.00	47.90	1SG 435
	ATOM	435	C	LEU	55	45.158	-18.260	19.279	1.00	47.90	1SG 436
	ATOM	436	O	LEU	55	46.036	-18.923	19.824	1.00	47.90	1SG 437
	ATOM	437	N	GLY	56	45.440	-17.393	18.292	1.00	45.33	1SG 438
	ATOM	438	CA	GLY	56	46.796	-17.227	17.861	1.00	45.33	1SG 439
	ATOM	439	C	GLY	56	47.057	-15.762	17.864	1.00	45.33	1SG 440
	ATOM	440	O	GLY	56	46.166	-14.961	18.144	1.00	45.33	1SG 441
	ATOM	441	N	ILE	57	48.299	-15.360	17.551	1.00	42.94	1SG 442
	ATOM	442	CA	ILE	57	48.540	-13.953	17.490	1.00	42.94	1SG 443

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	443	CB	ILE	57	49.902	-13.530	17.961	1.00	42.94	1SG 444
ATOM	444	CG2	ILE	57	50.962	-14.151	17.037	1.00	42.94	1SG 445
ATOM	445	CG1	ILE	57	49.969	-11.996	18.050	1.00	42.94	1SG 446
ATOM	446	CD1	ILE	57	51.202	-11.475	18.785	1.00	42.94	1SG 447
ATOM	447	C	ILE	57	48.424	-13.603	16.048	1.00	42.94	1SG 448
ATOM	448	O	ILE	57	48.944	-14.309	15.187	1.00	42.94	1SG 449
ATOM	449	N	GLN	58	47.687	-12.526	15.725	1.00	39.72	1SG 450
ATOM	450	CA	GLN	58	47.564	-12.239	14.332	1.00	39.72	1SG 451
ATOM	451	CB	GLN	58	46.525	-11.167	13.966	1.00	39.72	1SG 452
ATOM	452	CG	GLN	58	46.515	-10.850	12.466	1.00	39.72	1SG 453
ATOM	453	CD	GLN	58	46.163	-12.118	11.696	1.00	39.72	1SG 454
ATOM	454	OE1	GLN	58	45.085	-12.687	11.859	1.00	39.72	1SG 455
ATOM	455	NE2	GLN	58	47.104	-12.581	10.829	1.00	39.72	1SG 456
ATOM	456	C	GLN	58	48.888	-11.777	13.829	1.00	39.72	1SG 457
ATOM	457	O	GLN	58	49.555	-10.951	14.449	1.00	39.72	1SG 458
ATOM	458	N	GLY	59	49.309	-12.336	12.680	1.00	36.04	1SG 459
ATOM	459	CA	GLY	59	50.528	-11.910	12.064	1.00	36.04	1SG 460
ATOM	460	C	GLY	59	51.687	-12.639	12.660	1.00	36.04	1SG 461
ATOM	461	O	GLY	59	52.835	-12.353	12.325	1.00	36.04	1SG 462
ATOM	462	N	GLY	60	51.433	-13.609	13.556	1.00	35.52	1SG 463
ATOM	463	CA	GLY	60	52.554	-14.299	14.117	1.00	35.52	1SG 464
ATOM	464	C	GLY	60	52.189	-15.737	14.198	1.00	35.52	1SG 465
ATOM	465	O	GLY	60	51.033	-16.085	14.437	1.00	35.52	1SG 466
ATOM	466	N	SER	61	53.182	-16.623	14.005	1.00	38.49	1SG 467
ATOM	467	CA	SER	61	52.858	-18.008	14.106	1.00	38.49	1SG 468
ATOM	468	CB	SER	61	53.770	-18.906	13.254	1.00	38.49	1SG 469
ATOM	469	OG	SER	61	53.623	-18.588	11.878	1.00	38.49	1SG 470
ATOM	470	C	SER	61	53.090	-18.369	15.533	1.00	38.49	1SG 471
ATOM	471	O	SER	61	54.032	-19.091	15.857	1.00	38.49	1SG 472
ATOM	472	N	ARG	62	52.209	-17.869	16.423	1.00	41.73	1SG 473

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	473	CA	ARG	62	52.295	-18.162	17.823	1.00	41.73	1SG 474
	ATOM	474	CB	ARG	62	52.729	-16.964	18.687	1.00	41.73	1SG 475
	ATOM	475	CG	ARG	62	54.095	-16.379	18.317	1.00	41.73	1SG 476
	ATOM	476	CD	ARG	62	54.091	-15.611	16.993	1.00	41.73	1SG 477
	ATOM	477	NE	ARG	62	55.406	-14.921	16.859	1.00	41.73	1SG 478
	ATOM	478	CZ	ARG	62	56.461	-15.547	16.262	1.00	41.73	1SG 479
	ATOM	479	NH1	ARG	62	56.317	-16.810	15.766	1.00	41.73	1SG 480
	ATOM	480	NH2	ARG	62	57.661	-14.905	16.157	1.00	41.73	1SG 481
	ATOM	481	C	ARG	62	50.903	-18.503	18.245	1.00	41.73	1SG 482
	ATOM	482	O	ARG	62	49.960	-17.783	17.916	1.00	41.73	1SG 483
	ATOM	483	N	CYS	63	50.731	-19.606	19.000	1.00	43.44	1SG 484
	ATOM	484	CA	CYS	63	49.397	-20.008	19.331	1.00	43.44	1SG 485
	ATOM	485	CB	CYS	63	48.996	-21.247	18.523	1.00	43.44	1SG 486
	ATOM	486	SG	CYS	63	49.453	-21.047	16.773	1.00	43.44	1SG 487
	ATOM	487	C	CYS	63	49.357	-20.396	20.775	1.00	43.44	1SG 488
	ATOM	488	O	CYS	63	50.329	-20.923	21.314	1.00	43.44	1SG 489
	ATOM	489	N	LEU	64	48.222	-20.116	21.447	1.00	42.33	1SG 490
	ATOM	490	CA	LEU	64	48.069	-20.520	22.816	1.00	42.33	1SG 491
	ATOM	491	CB	LEU	64	46.996	-19.722	23.580	1.00	42.33	1SG 492
	ATOM	492	CG	LEU	64	47.413	-18.265	23.875	1.00	42.33	1SG 493
	ATOM	493	CD2	LEU	64	46.354	-17.546	24.727	1.00	42.33	1SG 494
	ATOM	494	CD1	LEU	64	47.745	-17.495	22.588	1.00	42.33	1SG 495
	ATOM	495	C	LEU	64	47.698	-21.964	22.792	1.00	42.33	1SG 496
	ATOM	496	O	LEU	64	46.903	-22.399	21.960	1.00	42.33	1SG 497
	ATOM	497	N	ALA	65	48.290	-22.752	23.708	1.00	36.60	1SG 498
	ATOM	498	CA	ALA	65	48.009	-24.154	23.756	1.00	36.60	1SG 499
	ATOM	499	CB	ALA	65	49.046	-25.014	23.014	1.00	36.60	1SG 500
	ATOM	500	C	ALA	65	48.059	-24.542	25.192	1.00	36.60	1SG 501
	ATOM	501	O	ALA	65	48.600	-23.810	26.021	1.00	36.60	1SG 502
	ATOM	502	N	CYS	66	47.452	-25.699	25.518	1.00	33.08	1SG 503

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	503	CA	CYS	66	47.452	-26.177	26.866	1.00	33.08	1SG 504
	ATOM	504	CB	CYS	66	46.041	-26.446	27.417	1.00	33.08	1SG 505
5	ATOM	505	SG	CYS	66	45.034	-24.934	27.501	1.00	33.08	1SG 506
	ATOM	506	C	CYS	66	48.185	-27.477	26.864	1.00	33.08	1SG 507
	ATOM	507	O	CYS	66	47.890	-28.366	26.067	1.00	33.08	1SG 508
	ATOM	508	N	VAL	67	49.186	-27.612	27.753	1.00	34.93	1SG 509
	ATOM	509	CA	VAL	67	49.923	-28.838	27.808	1.00	34.93	1SG 510
10	ATOM	510	CB	VAL	67	51.213	-28.794	27.043	1.00	34.93	1SG 511
	ATOM	511	CG1	VAL	67	50.893	-28.589	25.553	1.00	34.93	1SG 512
	ATOM	512	CG2	VAL	67	52.101	-27.692	27.647	1.00	34.93	1SG 513
	ATOM	513	C	VAL	67	50.267	-29.077	29.235	1.00	34.93	1SG 514
	ATOM	514	O	VAL	67	50.301	-28.149	30.043	1.00	34.93	1SG 515
15	ATOM	515	N	GLU	68	50.498	-30.351	29.594	1.00	41.24	1SG 516
	ATOM	516	CA	GLU	68	50.867	-30.624	30.948	1.00	41.24	1SG 517
	ATOM	517	CB	GLU	68	50.712	-32.106	31.332	1.00	41.24	1SG 518
	ATOM	518	CG	GLU	68	49.262	-32.595	31.320	1.00	41.24	1SG 519
	ATOM	519	CD	GLU	68	49.260	-34.065	31.715	1.00	41.24	1SG 520
20	ATOM	520	OE1	GLU	68	50.363	-34.602	32.000	1.00	41.24	1SG 521
	ATOM	521	OE2	GLU	68	48.155	-34.670	31.735	1.00	41.24	1SG 522
	ATOM	522	C	GLU	68	52.311	-30.275	31.097	1.00	41.24	1SG 523
	ATOM	523	O	GLU	68	53.151	-30.697	30.304	1.00	41.24	1SG 524
	ATOM	524	N	THR	69	52.630	-29.466	32.121	1.00	50.56	1SG 525
25	ATOM	525	CA	THR	69	53.984	-29.153	32.459	1.00	50.56	1SG 526
	ATOM	526	CB	THR	69	54.315	-27.686	32.351	1.00	50.56	1SG 527
	ATOM	527	OG1	THR	69	55.673	-27.464	32.698	1.00	50.56	1SG 528
	ATOM	528	CG2	THR	69	53.380	-26.864	33.256	1.00	50.56	1SG 529
	ATOM	529	C	THR	69	54.053	-29.579	33.876	1.00	50.56	1SG 530
30	ATOM	530	O	THR	69	53.165	-29.212	34.645	1.00	50.56	1SG 531
	ATOM	531	N	GLU	70	55.092	-30.365	34.244	1.00	57.23	1SG 532
	ATOM	532	CA	GLU	70	55.110	-30.985	35.537	1.00	57.23	1SG 533

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	533	CB	GLU	70	55.426	-30.052	36.740	1.00	57.23	1SG 534
ATOM	534	CG	GLU	70	54.504	-28.854	36.993	1.00	57.23	1SG 535
ATOM	535	CD	GLU	70	53.404	-29.266	37.962	1.00	57.23	1SG 536
ATOM	536	OE1	GLU	70	53.653	-30.184	38.788	1.00	57.23	1SG 537
ATOM	537	OE2	GLU	70	52.304	-28.654	37.898	1.00	57.23	1SG 538
ATOM	538	C	GLU	70	53.786	-31.666	35.622	1.00	57.23	1SG 539
ATOM	539	O	GLU	70	53.264	-32.104	34.599	1.00	57.23	1SG 540
ATOM	540	N	GLU	71	53.197	-31.836	36.806	1.00	58.06	1SG 541
ATOM	541	CA	GLU	71	51.892	-32.404	36.703	1.00	58.06	1SG 542
ATOM	542	CB	GLU	71	51.569	-33.403	37.826	1.00	58.06	1SG 543
ATOM	543	CG	GLU	71	50.280	-34.191	37.593	1.00	58.06	1SG 544
ATOM	544	CD	GLU	71	49.097	-33.288	37.912	1.00	58.06	1SG 545
ATOM	545	OE1	GLU	71	49.106	-32.668	39.009	1.00	58.06	1SG 546
ATOM	546	OE2	GLU	71	48.172	-33.202	37.062	1.00	58.06	1SG 547
ATOM	547	C	GLU	71	50.927	-31.267	36.792	1.00	58.06	1SG 548
ATOM	548	O	GLU	71	50.757	-30.671	37.854	1.00	58.06	1SG 549
ATOM	549	N	GLY	72	50.276	-30.917	35.664	1.00	53.24	1SG 550
ATOM	550	CA	GLY	72	49.310	-29.860	35.731	1.00	53.24	1SG 551
ATOM	551	C	GLY	72	49.173	-29.228	34.383	1.00	53.24	1SG 552
ATOM	552	O	GLY	72	50.135	-29.096	33.626	1.00	53.24	1SG 553
ATOM	553	N	PRO	73	47.968	-28.818	34.085	1.00	47.47	1SG 554
ATOM	554	CA	PRO	73	47.742	-28.160	32.833	1.00	47.47	1SG 555
ATOM	555	CD	PRO	73	46.807	-29.585	34.507	1.00	47.47	1SG 556
ATOM	556	CB	PRO	73	46.232	-28.164	32.618	1.00	47.47	1SG 557
ATOM	557	CG	PRO	73	45.773	-29.421	33.380	1.00	47.47	1SG 558
ATOM	558	C	PRO	73	48.355	-26.806	32.891	1.00	47.47	1SG 559
ATOM	559	O	PRO	73	48.345	-26.189	33.956	1.00	47.47	1SG 560
ATOM	560	N	SER	74	48.895	-26.325	31.760	1.00	42.89	1SG 561
ATOM	561	CA	SER	74	49.528	-25.047	31.793	1.00	42.89	1SG 562
ATOM	562	CB	SER	74	51.040	-25.164	32.032	1.00	42.89	1SG 563

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	563	OG	SER	74	51.587	-23.893	32.328	1.00	42.89	1SG 564
ATOM	564	C	SER	74	49.301	-24.417	30.457	1.00	42.89	1SG 565
ATOM	565	O	SER	74	49.223	-25.108	29.441	1.00	42.89	1SG 566
ATOM	566	N	LEU	75	49.187	-23.074	30.434	1.00	39.97	1SG 567
ATOM	567	CA	LEU	75	48.941	-22.345	29.224	1.00	39.97	1SG 568
ATOM	568	CB	LEU	75	48.073	-21.096	29.458	1.00	39.97	1SG 569
ATOM	569	CG	LEU	75	47.778	-20.276	28.191	1.00	39.97	1SG 570
ATOM	570	CD2	LEU	75	47.229	-18.885	28.547	1.00	39.97	1SG 571
ATOM	571	CD1	LEU	75	46.879	-21.052	27.215	1.00	39.97	1SG 572
ATOM	572	C	LEU	75	50.264	-21.874	28.705	1.00	39.97	1SG 573
ATOM	573	O	LEU	75	51.063	-21.309	29.450	1.00	39.97	1SG 574
ATOM	574	N	GLN	76	50.542	-22.111	27.406	1.00	39.38	1SG 575
ATOM	575	CA	GLN	76	51.799	-21.660	26.877	1.00	39.38	1SG 576
ATOM	576	CB	GLN	76	52.863	-22.766	26.765	1.00	39.38	1SG 577
ATOM	577	CG	GLN	76	52.497	-23.847	25.744	1.00	39.38	1SG 578
ATOM	578	CD	GLN	76	53.654	-24.832	25.650	1.00	39.38	1SG 579
ATOM	579	OE1	GLN	76	54.517	-24.885	26.524	1.00	39.38	1SG 580
ATOM	580	NE2	GLN	76	53.675	-25.635	24.552	1.00	39.38	1SG 581
ATOM	581	C	GLN	76	51.578	-21.172	25.483	1.00	39.38	1SG 582
ATOM	582	O	GLN	76	50.531	-21.407	24.883	1.00	39.38	1SG 583
ATOM	583	N	LEU	77	52.575	-20.441	24.945	1.00	38.94	1SG 584
ATOM	584	CA	LEU	77	52.534	-19.988	23.584	1.00	38.94	1SG 585
ATOM	585	CB	LEU	77	53.069	-18.560	23.372	1.00	38.94	1SG 586
ATOM	586	CG	LEU	77	52.141	-17.462	23.921	1.00	38.94	1SG 587
ATOM	587	CD2	LEU	77	51.833	-17.693	25.405	1.00	38.94	1SG 588
ATOM	588	CD1	LEU	77	50.862	-17.334	23.078	1.00	38.94	1SG 589
ATOM	589	C	LEU	77	53.432	-20.915	22.841	1.00	38.94	1SG 590
ATOM	590	O	LEU	77	54.532	-21.220	23.301	1.00	38.94	1SG 591
ATOM	591	N	GLU	78	52.973	-21.405	21.674	1.00	36.56	1SG 592
ATOM	592	CA	GLU	78	53.755	-22.355	20.940	1.00	36.56	1SG 593

		Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	593	CB	GLU	78	53.041	-23.714	20.838	1.00	36.56	1SG 594
	ATOM	594	CG	GLU	78	53.820	-24.812	20.116	1.00	36.56	1SG 595
5	ATOM	595	CD	GLU	78	52.942	-26.056	20.131	1.00	36.56	1SG 596
	ATOM	596	OE1	GLU	78	52.261	-26.280	21.167	1.00	36.56	1SG 597
	ATOM	597	OE2	GLU	78	52.933	-26.793	19.109	1.00	36.56	1SG 598
	ATOM	598	C	GLU	78	53.953	-21.829	19.555	1.00	36.56	1SG 599
	ATOM	599	O	GLU	78	53.044	-21.246	18.968	1.00	36.56	1SG 600
10	ATOM	600	N	ASP	79	55.179	-21.993	19.016	1.00	34.75	1SG 601
	ATOM	601	CA	ASP	79	55.477	-21.569	17.677	1.00	34.75	1SG 602
	ATOM	602	CB	ASP	79	56.985	-21.341	17.454	1.00	34.75	1SG 603
	ATOM	603	CG	ASP	79	57.208	-20.499	16.204	1.00	34.75	1SG 604
	ATOM	604	OD1	ASP	79	56.794	-20.935	15.097	1.00	34.75	1SG 605
15	ATOM	605	OD2	ASP	79	57.816	-19.404	16.343	1.00	34.75	1SG 606
	ATOM	606	C	ASP	79	55.017	-22.669	16.770	1.00	34.75	1SG 607
	ATOM	607	O	ASP	79	55.001	-23.835	17.163	1.00	34.75	1SG 608
	ATOM	608	N	VAL	80	54.622	-22.332	15.526	1.00	33.44	1SG 609
	ATOM	609	CA	VAL	80	54.154	-23.352	14.633	1.00	33.44	1SG 610
20	ATOM	610	CB	VAL	80	52.661	-23.465	14.631	1.00	33.44	1SG 611
	ATOM	611	CG1	VAL	80	52.215	-23.832	16.057	1.00	33.44	1SG 612
	ATOM	612	CG2	VAL	80	52.069	-22.141	14.117	1.00	33.44	1SG 613
	ATOM	613	C	VAL	80	54.573	-22.974	13.248	1.00	33.44	1SG 614
	ATOM	614	O	VAL	80	54.881	-21.815	12.979	1.00	33.44	1SG 615
25	ATOM	615	N	ASN	81	54.622	-23.958	12.327	1.00	34.97	1SG 616
	ATOM	616	CA	ASN	81	54.977	-23.616	10.981	1.00	34.97	1SG 617
	ATOM	617	CB	ASN	81	55.218	-24.837	10.075	1.00	34.97	1SG 618
	ATOM	618	CG	ASN	81	55.874	-24.360	8.785	1.00	34.97	1SG 619
	ATOM	619	OD1	ASN	81	57.010	-24.723	8.484	1.00	34.97	1SG 620
30	ATOM	620	ND2	ASN	81	55.146	-23.523	7.998	1.00	34.97	1SG 621
	ATOM	621	C	ASN	81	53.813	-22.853	10.443	1.00	34.97	1SG 622
	ATOM	622	O	ASN	81	52.678	-23.326	10.479	1.00	34.97	1SG 623

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	623	N	ILE	82	54.064	-21.631	9.939	1.00	40.29	1SG 624
ATOM	624	CA	ILE	82	52.968	-20.830	9.488	1.00	40.29	1SG 625
ATOM	625	CB	ILE	82	53.380	-19.422	9.143	1.00	40.29	1SG 626
ATOM	626	CG2	ILE	82	54.483	-19.461	8.072	1.00	40.29	1SG 627
ATOM	627	CG1	ILE	82	52.149	-18.583	8.762	1.00	40.29	1SG 628
ATOM	628	CD1	ILE	82	52.436	-17.085	8.674	1.00	40.29	1SG 629
ATOM	629	C	ILE	82	52.320	-21.446	8.290	1.00	40.29	1SG 630
ATOM	630	O	ILE	82	51.104	-21.625	8.263	1.00	40.29	1SG 631
ATOM	631	N	GLU	83	53.123	-21.797	7.271	1.00	52.05	1SG 632
ATOM	632	CA	GLU	83	52.595	-22.322	6.046	1.00	52.05	1SG 633
ATOM	633	CB	GLU	83	53.650	-22.382	4.928	1.00	52.05	1SG 634
ATOM	634	CG	GLU	83	53.087	-22.802	3.568	1.00	52.05	1SG 635
ATOM	635	CD	GLU	83	52.374	-21.601	2.960	1.00	52.05	1SG 636
ATOM	636	OE1	GLU	83	52.283	-20.554	3.654	1.00	52.05	1SG 637
ATOM	637	OE2	GLU	83	51.912	-21.716	1.793	1.00	52.05	1SG 638
ATOM	638	C	GLU	83	52.074	-23.710	6.227	1.00	52.05	1SG 639
ATOM	639	O	GLU	83	50.987	-24.040	5.753	1.00	52.05	1SG 640
ATOM	640	N	GLU	84	52.828	-24.556	6.951	1.00	66.46	1SG 641
ATOM	641	CA	GLU	84	52.492	-25.949	6.990	1.00	66.46	1SG 642
ATOM	642	CB	GLU	84	53.450	-26.752	7.884	1.00	66.46	1SG 643
ATOM	643	CG	GLU	84	54.885	-26.790	7.359	1.00	66.46	1SG 644
ATOM	644	CD	GLU	84	55.715	-27.604	8.340	1.00	66.46	1SG 645
ATOM	645	OE1	GLU	84	55.137	-28.066	9.360	1.00	66.46	1SG 646
ATOM	646	OE2	GLU	84	56.937	-27.774	8.085	1.00	66.46	1SG 647
ATOM	647	C	GLU	84	51.134	-26.133	7.547	1.00	66.46	1SG 648
ATOM	648	O	GLU	84	50.259	-26.744	6.934	1.00	66.46	1SG 649
ATOM	649	N	LEU	85	50.907	-25.581	8.735	1.00	76.27	1SG 650
ATOM	650	CA	LEU	85	49.617	-25.767	9.291	1.00	76.27	1SG 651
ATOM	651	CB	LEU	85	49.541	-26.969	10.256	1.00	76.27	1SG 652
ATOM	652	CG	LEU	85	48.119	-27.305	10.750	1.00	76.27	1SG 653

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	653	CD2	LEU	85	48.145	-28.388	11.841	1.00	76.27	1SG 654
	ATOM	654	CD1	LEU	85	47.197	-27.672	9.576	1.00	76.27	1SG 655
	ATOM	655	C	LEU	85	49.373	-24.520	10.039	1.00	76.27	1SG 656
	ATOM	656	O	LEU	85	50.142	-23.566	9.927	1.00	76.27	1SG 657
	ATOM	657	N	TYR	86	48.261	-24.493	10.779	1.00	82.94	1SG 658
	ATOM	658	CA	TYR	86	47.945	-23.392	11.618	1.00	82.94	1SG 659
	ATOM	659	CB	TYR	86	49.143	-22.743	12.330	1.00	82.94	1SG 660
	ATOM	660	CG	TYR	86	49.629	-23.839	13.212	1.00	82.94	1SG 661
	ATOM	661	CD1	TYR	86	50.528	-24.767	12.739	1.00	82.94	1SG 662
	ATOM	662	CD2	TYR	86	49.165	-23.960	14.500	1.00	82.94	1SG 663
	ATOM	663	CE1	TYR	86	50.970	-25.790	13.543	1.00	82.94	1SG 664
	ATOM	664	CE2	TYR	86	49.603	-24.981	15.310	1.00	82.94	1SG 665
	ATOM	665	CZ	TYR	86	50.508	-25.898	14.831	1.00	82.94	1SG 666
	ATOM	666	OH	TYR	86	50.962	-26.948	15.657	1.00	82.94	1SG 667
	ATOM	667	C	TYR	86	47.128	-22.412	10.883	1.00	82.94	1SG 668
	ATOM	668	O	TYR	86	47.412	-22.054	9.741	1.00	82.94	1SG 669
	ATOM	669	N	LYS	87	46.095	-21.917	11.583	1.00	77.48	1SG 670
	ATOM	670	CA	LYS	87	45.112	-21.117	10.945	1.00	77.48	1SG 671
	ATOM	671	CB	LYS	87	45.706	-19.940	10.153	1.00	77.48	1SG 672
	ATOM	672	CG	LYS	87	46.442	-18.916	11.022	1.00	77.48	1SG 673
	ATOM	673	CD	LYS	87	45.557	-18.238	12.070	1.00	77.48	1SG 674
	ATOM	674	CE	LYS	87	45.379	-19.058	13.349	1.00	77.48	1SG 675
	ATOM	675	NZ	LYS	87	46.642	-19.073	14.121	1.00	77.48	1SG 676
	ATOM	676	C	LYS	87	44.428	-22.028	9.987	1.00	77.48	1SG 677
	ATOM	677	O	LYS	87	44.182	-21.674	8.836	1.00	77.48	1SG 678
	ATOM	678	N	GLY	88	44.127	-23.262	10.449	1.00	67.17	1SG 679
	ATOM	679	CA	GLY	88	43.445	-24.165	9.576	1.00	67.17	1SG 680
	ATOM	680	C	GLY	88	42.471	-24.999	10.347	1.00	67.17	1SG 681
	ATOM	681	O	GLY	88	42.788	-25.521	11.415	1.00	67.17	1SG 682
	ATOM	682	N	GLY	89	41.225	-25.034	9.826	1.00	57.43	1SG 683

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	683	CA	GLY	89	40.112	-25.890	10.148	1.00	57.43	1SG 684
ATOM	684	C	GLY	89	40.328	-26.702	11.377	1.00	57.43	1SG 685
ATOM	685	O	GLY	89	40.056	-26.277	12.498	1.00	57.43	1SG 686
ATOM	686	N	GLU	90	40.797	-27.945	11.147	1.00	47.61	1SG 687
ATOM	687	CA	GLU	90	41.017	-28.906	12.182	1.00	47.61	1SG 688
ATOM	688	CB	GLU	90	41.067	-30.344	11.640	1.00	47.61	1SG 689
ATOM	689	CG	GLU	90	41.181	-31.433	12.706	1.00	47.61	1SG 690
ATOM	690	CD	GLU	90	41.212	-32.768	11.973	1.00	47.61	1SG 691
ATOM	691	OE1	GLU	90	41.160	-32.748	10.714	1.00	47.61	1SG 692
ATOM	692	OE2	GLU	90	41.287	-33.823	12.658	1.00	47.61	1SG 693
ATOM	693	C	GLU	90	42.329	-28.598	12.813	1.00	47.61	1SG 694
ATOM	694	O	GLU	90	43.387	-28.752	12.205	1.00	47.61	1SG 695
ATOM	695	N	GLU	91	42.277	-28.162	14.081	1.00	42.98	1SG 696
ATOM	696	CA	GLU	91	43.457	-27.795	14.796	1.00	42.98	1SG 697
ATOM	697	CB	GLU	91	43.326	-26.435	15.504	1.00	42.98	1SG 698
ATOM	698	CG	GLU	91	44.640	-25.896	16.070	1.00	42.98	1SG 699
ATOM	699	CD	GLU	91	45.438	-25.320	14.909	1.00	42.98	1SG 700
ATOM	700	OE1	GLU	91	44.969	-25.457	13.748	1.00	42.98	1SG 701
ATOM	701	OE2	GLU	91	46.523	-24.735	15.167	1.00	42.98	1SG 702
ATOM	702	C	GLU	91	43.646	-28.831	15.853	1.00	42.98	1SG 703
ATOM	703	O	GLU	91	42.695	-29.503	16.248	1.00	42.98	1SG 704
ATOM	704	N	ALA	92	44.894	-28.996	16.327	1.00	42.31	1SG 705
ATOM	705	CA	ALA	92	45.159	-29.975	17.340	1.00	42.31	1SG 706
ATOM	706	CB	ALA	92	46.646	-30.094	17.712	1.00	42.31	1SG 707
ATOM	707	C	ALA	92	44.409	-29.561	18.569	1.00	42.31	1SG 708
ATOM	708	O	ALA	92	44.121	-28.383	18.767	1.00	42.31	1SG 709
ATOM	709	N	THR	93	44.086	-30.545	19.430	1.00	43.29	1SG 710
ATOM	710	CA	THR	93	43.271	-30.374	20.603	1.00	43.29	1SG 711
ATOM	711	CB	THR	93	43.007	-31.665	21.318	1.00	43.29	1SG 712
ATOM	712	OG1	THR	93	42.362	-32.583	20.448	1.00	43.29	1SG 713

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	713	CG2	THR	93	42.111	-31.375	22.535	1.00	43.29	1SG 714
ATOM	714	C	THR	93	43.901	-29.442	21.594	1.00	43.29	1SG 715
ATOM	715	O	THR	93	43.196	-28.699	22.275	1.00	43.29	1SG 716
ATOM	716	N	ARG	94	45.238	-29.448	21.718	1.00	46.17	1SG 717
ATOM	717	CA	ARG	94	45.875	-28.636	22.719	1.00	46.17	1SG 718
ATOM	718	CB	ARG	94	47.406	-28.811	22.766	1.00	46.17	1SG 719
ATOM	719	CG	ARG	94	48.142	-28.428	21.480	1.00	46.17	1SG 720
ATOM	720	CD	ARG	94	49.655	-28.655	21.568	1.00	46.17	1SG 721
ATOM	721	NE	ARG	94	50.260	-28.233	20.274	1.00	46.17	1SG 722
ATOM	722	CZ	ARG	94	50.342	-29.108	19.230	1.00	46.17	1SG 723
ATOM	723	NH1	ARG	94	49.860	-30.378	19.363	1.00	46.17	1SG 724
ATOM	724	NH2	ARG	94	50.904	-28.711	18.051	1.00	46.17	1SG 725
ATOM	725	C	ARG	94	45.566	-27.192	22.454	1.00	46.17	1SG 726
ATOM	726	O	ARG	94	45.525	-26.375	23.374	1.00	46.17	1SG 727
ATOM	727	N	PHE	95	45.403	-26.843	21.168	1.00	49.82	1SG 728
ATOM	728	CA	PHE	95	45.133	-25.514	20.689	1.00	49.82	1SG 729
ATOM	729	CB	PHE	95	45.411	-25.340	19.187	1.00	49.82	1SG 730
ATOM	730	CG	PHE	95	46.884	-25.457	18.977	1.00	49.82	1SG 731
ATOM	731	CD1	PHE	95	47.718	-24.401	19.266	1.00	49.82	1SG 732
ATOM	732	CD2	PHE	95	47.435	-26.628	18.511	1.00	49.82	1SG 733
ATOM	733	CE1	PHE	95	49.077	-24.505	19.077	1.00	49.82	1SG 734
ATOM	734	CE2	PHE	95	48.792	-26.737	18.319	1.00	49.82	1SG 735
ATOM	735	CZ	PHE	95	49.616	-25.675	18.601	1.00	49.82	1SG 736
ATOM	736	C	PHE	95	43.719	-25.050	20.934	1.00	49.82	1SG 737
ATOM	737	O	PHE	95	43.466	-23.845	20.913	1.00	49.82	1SG 738
ATOM	738	N	THR	96	42.744	-25.971	21.091	1.00	48.19	1SG 739
ATOM	739	CA	THR	96	41.360	-25.568	21.131	1.00	48.19	1SG 740
ATOM	740	CB	THR	96	40.427	-26.623	20.609	1.00	48.19	1SG 741
ATOM	741	OG1	THR	96	40.747	-26.939	19.262	1.00	48.19	1SG 742
ATOM	742	CG2	THR	96	38.985	-26.096	20.702	1.00	48.19	1SG 743

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	743	C	THR	96	40.872	-25.206	22.502	1.00	48.19	1SG 744
	ATOM	744	O	THR	96	41.198	-25.835	23.509	1.00	48.19	1SG 745
	ATOM	745	N	PHE	97	40.043	-24.139	22.537	1.00	46.05	1SG 746
	ATOM	746	CA	PHE	97	39.392	-23.669	23.724	1.00	46.05	1SG 747
	ATOM	747	CB	PHE	97	39.817	-22.253	24.158	1.00	46.05	1SG 748
	ATOM	748	CG	PHE	97	41.227	-22.296	24.639	1.00	46.05	1SG 749
	ATOM	749	CD1	PHE	97	42.279	-22.175	23.760	1.00	46.05	1SG 750
	ATOM	750	CD2	PHE	97	41.496	-22.454	25.979	1.00	46.05	1SG 751
	ATOM	751	CE1	PHE	97	43.578	-22.213	24.212	1.00	46.05	1SG 752
	ATOM	752	CE2	PHE	97	42.792	-22.493	26.437	1.00	46.05	1SG 753
	ATOM	753	CZ	PHE	97	43.837	-22.373	25.553	1.00	46.05	1SG 754
	ATOM	754	C	PHE	97	37.938	-23.588	23.388	1.00	46.05	1SG 755
	ATOM	755	O	PHE	97	37.563	-23.348	22.241	1.00	46.05	1SG 756
	ATOM	756	N	PHE	98	37.066	-23.812	24.384	1.00	42.94	1SG 757
	ATOM	757	CA	PHE	98	35.665	-23.708	24.119	1.00	42.94	1SG 758
	ATOM	758	CB	PHE	98	34.816	-24.783	24.819	1.00	42.94	1SG 759
	ATOM	759	CG	PHE	98	35.051	-26.089	24.143	1.00	42.94	1SG 760
	ATOM	760	CD1	PHE	98	36.159	-26.849	24.439	1.00	42.94	1SG 761
	ATOM	761	CD2	PHE	98	34.149	-26.558	23.217	1.00	42.94	1SG 762
	ATOM	762	CE1	PHE	98	36.364	-28.055	23.812	1.00	42.94	1SG 763
	ATOM	763	CE2	PHE	98	34.350	-27.763	22.587	1.00	42.94	1SG 764
	ATOM	764	CZ	PHE	98	35.460	-28.515	22.885	1.00	42.94	1SG 765
	ATOM	765	C	PHE	98	35.235	-22.390	24.656	1.00	42.94	1SG 766
	ATOM	766	O	PHE	98	35.414	-22.108	25.840	1.00	42.94	1SG 767
	ATOM	767	N	GLN	99	34.676	-21.537	23.775	1.00	39.29	1SG 768
	ATOM	768	CA	GLN	99	34.184	-20.276	24.227	1.00	39.29	1SG 769
	ATOM	769	CB	GLN	99	34.032	-19.210	23.126	1.00	39.29	1SG 770
	ATOM	770	CG	GLN	99	35.359	-18.700	22.563	1.00	39.29	1SG 771
	ATOM	771	CD	GLN	99	35.045	-17.660	21.495	1.00	39.29	1SG 772
	ATOM	772	OE1	GLN	99	33.988	-17.693	20.867	1.00	39.29	1SG 773

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	773	NE2	GLN	99	35.988	-16.703	21.286	1.00	39.29	1SG 774
ATOM	774	C	GLN	99	32.822	-20.554	24.765	1.00	39.29	1SG 775
ATOM	775	O	GLN	99	31.971	-21.115	24.075	1.00	39.29	1SG 776
ATOM	776	N	SER	100	32.591	-20.175	26.035	1.00	38.03	1SG 777
ATOM	777	CA	SER	100	31.320	-20.430	26.639	1.00	38.03	1SG 778
ATOM	778	CB	SER	100	31.415	-21.217	27.957	1.00	38.03	1SG 779
ATOM	779	OG	SER	100	32.107	-20.453	28.933	1.00	38.03	1SG 780
ATOM	780	C	SER	100	30.714	-19.111	26.961	1.00	38.03	1SG 781
ATOM	781	O	SER	100	31.403	-18.175	27.366	1.00	38.03	1SG 782
ATOM	782	N	SER	101	29.388	-19.005	26.776	1.00	36.29	1SG 783
ATOM	783	CA	SER	101	28.752	-17.757	27.056	1.00	36.29	1SG 784
ATOM	784	CB	SER	101	27.779	-17.308	25.954	1.00	36.29	1SG 785
ATOM	785	OG	SER	101	26.695	-18.221	25.862	1.00	36.29	1SG 786
ATOM	786	C	SER	101	27.944	-17.933	28.293	1.00	36.29	1SG 787
ATOM	787	O	SER	101	27.096	-18.820	28.375	1.00	36.29	1SG 788
ATOM	788	N	SER	102	28.214	-17.086	29.302	1.00	34.22	1SG 789
ATOM	789	CA	SER	102	27.433	-17.099	30.499	1.00	34.22	1SG 790
ATOM	790	CB	SER	102	28.186	-17.663	31.718	1.00	34.22	1SG 791
ATOM	791	OG	SER	102	29.341	-16.883	31.994	1.00	34.22	1SG 792
ATOM	792	C	SER	102	27.113	-15.667	30.758	1.00	34.22	1SG 793
ATOM	793	O	SER	102	27.991	-14.876	31.098	1.00	34.22	1SG 794
ATOM	794	N	GLY	103	25.835	-15.286	30.599	1.00	32.47	1SG 795
ATOM	795	CA	GLY	103	25.530	-13.900	30.767	1.00	32.47	1SG 796
ATOM	796	C	GLY	103	26.265	-13.212	29.663	1.00	32.47	1SG 797
ATOM	797	O	GLY	103	26.507	-13.794	28.609	1.00	32.47	1SG 798
ATOM	798	N	SER	104	26.641	-11.942	29.874	1.00	33.67	1SG 799
ATOM	799	CA	SER	104	27.366	-11.220	28.871	1.00	33.67	1SG 800
ATOM	800	CB	SER	104	27.414	-9.708	29.149	1.00	33.67	1SG 801
ATOM	801	OG	SER	104	28.126	-9.041	28.117	1.00	33.67	1SG 802
ATOM	802	C	SER	104	28.778	-11.725	28.850	1.00	33.67	1SG 803

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	803	O	SER	104	29.528	-11.461	27.911	1.00	33.67	1SG 804
	ATOM	804	N	ALA	105	29.170	-12.452	29.914	1.00	36.18	1SG 805
5	ATOM	805	CA	ALA	105	30.512	-12.932	30.118	1.00	36.18	1SG 806
	ATOM	806	CB	ALA	105	30.761	-13.358	31.571	1.00	36.18	1SG 807
	ATOM	807	C	ALA	105	30.823	-14.127	29.274	1.00	36.18	1SG 808
	ATOM	808	O	ALA	105	29.941	-14.891	28.883	1.00	36.18	1SG 809
	ATOM	809	N	PHE	106	32.129	-14.309	28.973	1.00	40.27	1SG 810
10	ATOM	810	CA	PHE	106	32.558	-15.447	28.217	1.00	40.27	1SG 811
	ATOM	811	CB	PHE	106	32.928	-15.094	26.770	1.00	40.27	1SG 812
	ATOM	812	CG	PHE	106	31.607	-14.775	26.161	1.00	40.27	1SG 813
	ATOM	813	CD1	PHE	106	31.001	-13.560	26.389	1.00	40.27	1SG 814
	ATOM	814	CD2	PHE	106	30.966	-15.701	25.372	1.00	40.27	1SG 815
15	ATOM	815	CE1	PHE	106	29.775	-13.274	25.835	1.00	40.27	1SG 816
	ATOM	816	CE2	PHE	106	29.741	-15.421	24.815	1.00	40.27	1SG 817
	ATOM	817	CZ	PHE	106	29.144	-14.206	25.048	1.00	40.27	1SG 818
	ATOM	818	C	PHE	106	33.720	-16.070	28.920	1.00	40.27	1SG 819
	ATOM	819	O	PHE	106	34.522	-15.384	29.553	1.00	40.27	1SG 820
20	ATOM	820	N	ARG	107	33.810	-17.413	28.849	1.00	44.06	1SG 821
	ATOM	821	CA	ARG	107	34.882	-18.121	29.482	1.00	44.06	1SG 822
	ATOM	822	CB	ARG	107	34.434	-19.063	30.617	1.00	44.06	1SG 823
	ATOM	823	CG	ARG	107	33.858	-18.338	31.834	1.00	44.06	1SG 824
	ATOM	824	CD	ARG	107	32.483	-17.715	31.582	1.00	44.06	1SG 825
25	ATOM	825	NE	ARG	107	31.496	-18.830	31.518	1.00	44.06	1SG 826
	ATOM	826	CZ	ARG	107	30.924	-19.303	32.664	1.00	44.06	1SG 827
	ATOM	827	NH1	ARG	107	31.244	-18.745	33.868	1.00	44.06	1SG 828
	ATOM	828	NH2	ARG	107	30.030	-20.333	32.606	1.00	44.06	1SG 829
	ATOM	829	C	ARG	107	35.510	-18.976	28.432	1.00	44.06	1SG 830
30	ATOM	830	O	ARG	107	34.829	-19.459	27.527	1.00	44.06	1SG 831
	ATOM	831	N	LEU	108	36.843	-19.155	28.524	1.00	43.88	1SG 832
	ATOM	832	CA	LEU	108	37.561	-19.972	27.588	1.00	43.88	1SG 833

	Atom		Amino Acid		X	Y	Z	Occup.	B Factor	
	No.	Name	SC	No.						
ATOM	833	CB	LEU	108	38.797	-19.271	26.999	1.00	43.88	1SG 834
ATOM	834	CG	LEU	108	38.462	-18.001	26.194	1.00	43.88	1SG 835
ATOM	835	CD2	LEU	108	39.691	-17.485	25.428	1.00	43.88	1SG 836
ATOM	836	CD1	LEU	108	37.827	-16.924	27.089	1.00	43.88	1SG 837
ATOM	837	C	LEU	108	38.051	-21.170	28.333	1.00	43.88	1SG 838
ATOM	838	O	LEU	108	38.964	-21.087	29.154	1.00	43.88	1SG 839
ATOM	839	N	GLU	109	37.453	-22.335	28.038	1.00	41.59	1SG 840
ATOM	840	CA	GLU	109	37.797	-23.555	28.713	1.00	41.59	1SG 841
ATOM	841	CB	GLU	109	36.562	-24.411	29.029	1.00	41.59	1SG 842
ATOM	842	CG	GLU	109	36.881	-25.781	29.624	1.00	41.59	1SG 843
ATOM	843	CD	GLU	109	35.600	-26.599	29.563	1.00	41.59	1SG 844
ATOM	844	OE1	GLU	109	34.814	-26.545	30.546	1.00	41.59	1SG 845
ATOM	845	OE2	GLU	109	35.384	-27.284	28.527	1.00	41.59	1SG 846
ATOM	846	C	GLU	109	38.633	-24.369	27.786	1.00	41.59	1SG 847
ATOM	847	O	GLU	109	38.373	-24.419	26.586	1.00	41.59	1SG 848
ATOM	848	N	ALA	110	39.676	-25.029	28.324	1.00	38.02	1SG 849
ATOM	849	CA	ALA	110	40.494	-25.850	27.482	1.00	38.02	1SG 850
ATOM	850	CB	ALA	110	41.774	-26.354	28.170	1.00	38.02	1SG 851
ATOM	851	C	ALA	110	39.692	-27.049	27.078	1.00	38.02	1SG 852
ATOM	852	O	ALA	110	38.991	-27.648	27.893	1.00	38.02	1SG 853
ATOM	853	N	ALA	111	39.769	-27.408	25.779	1.00	37.70	1SG 854
ATOM	854	CA	ALA	111	39.096	-28.566	25.268	1.00	37.70	1SG 855
ATOM	855	CB	ALA	111	39.238	-28.710	23.744	1.00	37.70	1SG 856
ATOM	856	C	ALA	111	39.743	-29.761	25.887	1.00	37.70	1SG 857
ATOM	857	O	ALA	111	39.080	-30.730	26.253	1.00	37.70	1SG 858
ATOM	858	N	ALA	112	41.086	-29.710	25.985	1.00	42.33	1SG 859
ATOM	859	CA	ALA	112	41.885	-30.783	26.499	1.00	42.33	1SG 860
ATOM	860	CB	ALA	112	43.393	-30.506	26.373	1.00	42.33	1SG 861
ATOM	861	C	ALA	112	41.601	-31.026	27.950	1.00	42.33	1SG 862
ATOM	862	O	ALA	112	41.457	-32.176	28.363	1.00	42.33	1SG 863

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	863	N	TRP	113	41.523	-29.959	28.773	1.00	51.12	1SG 864
ATOM	864	CA	TRP	113	41.267	-30.162	30.173	1.00	51.12	1SG 865
ATOM	865	CB	TRP	113	42.358	-29.572	31.077	1.00	51.12	1SG 866
ATOM	866	CG	TRP	113	43.692	-30.261	30.926	1.00	51.12	1SG 867
ATOM	867	CD2	TRP	113	44.098	-31.408	31.688	1.00	51.12	1SG 868
ATOM	868	CD1	TRP	113	44.725	-29.970	30.084	1.00	51.12	1SG 869
ATOM	869	NE1	TRP	113	45.750	-30.866	30.272	1.00	51.12	1SG 870
ATOM	870	CE2	TRP	113	45.377	-31.757	31.257	1.00	51.12	1SG 871
ATOM	871	CE3	TRP	113	43.456	-32.113	32.665	1.00	51.12	1SG 872
ATOM	872	CZ2	TRP	113	46.038	-32.822	31.800	1.00	51.12	1SG 873
ATOM	873	CZ3	TRP	113	44.125	-33.186	33.211	1.00	51.12	1SG 874
ATOM	874	CH2	TRP	113	45.391	-33.533	32.787	1.00	51.12	1SG 875
ATOM	875	C	TRP	113	39.992	-29.445	30.486	1.00	51.12	1SG 876
ATOM	876	O	TRP	113	39.952	-28.217	30.522	1.00	51.12	1SG 877
ATOM	877	N	PRO	114	38.959	-30.188	30.772	1.00	61.83	1SG 878
ATOM	878	CA	PRO	114	37.661	-29.591	30.937	1.00	61.83	1SG 879
ATOM	879	CD	PRO	114	38.865	-31.550	30.271	1.00	61.83	1SG 880
ATOM	880	CB	PRO	114	36.677	-30.757	30.989	1.00	61.83	1SG 881
ATOM	881	CG	PRO	114	37.359	-31.833	30.123	1.00	61.83	1SG 882
ATOM	882	C	PRO	114	37.472	-28.574	32.022	1.00	61.83	1SG 883
ATOM	883	O	PRO	114	36.697	-27.644	31.848	1.00	61.83	1SG 884
ATOM	884	N	GLY	115	38.097	-28.722	33.181	1.00	68.27	1SG 885
ATOM	885	CA	GLY	115	37.874	-27.769	34.229	1.00	68.27	1SG 886
ATOM	886	C	GLY	115	38.590	-26.478	34.011	1.00	68.27	1SG 887
ATOM	887	O	GLY	115	38.211	-25.458	34.581	1.00	68.27	1SG 888
ATOM	888	N	TRP	116	39.704	-26.522	33.263	1.00	68.48	1SG 889
ATOM	889	CA	TRP	116	40.629	-25.426	33.200	1.00	68.48	1SG 890
ATOM	890	CB	TRP	116	42.011	-25.937	32.794	1.00	68.48	1SG 891
ATOM	891	CG	TRP	116	42.440	-27.016	33.752	1.00	68.48	1SG 892
ATOM	892	CD2	TRP	116	43.383	-26.852	34.820	1.00	68.48	1SG 893

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	893	CD1	TRP	116	42.011	-28.309	33.807	1.00	68.48	1SG 894
ATOM	894	NE1	TRP	116	42.618	-28.960	34.852	1.00	68.48	1SG 895
ATOM	895	CE2	TRP	116	43.469	-28.076	35.482	1.00	68.48	1SG 896
ATOM	896	CE3	TRP	116	44.114	-25.770	35.219	1.00	68.48	1SG 897
ATOM	897	CZ2	TRP	116	44.292	-28.237	36.560	1.00	68.48	1SG 898
ATOM	898	CZ3	TRP	116	44.947	-25.937	36.303	1.00	68.48	1SG 899
ATOM	899	CH2	TRP	116	45.034	-27.147	36.960	1.00	68.48	1SG 900
ATOM	900	C	TRP	116	40.205	-24.355	32.252	1.00	68.48	1SG 901
ATOM	901	O	TRP	116	39.756	-24.628	31.139	1.00	68.48	1SG 902
ATOM	902	N	PHE	117	40.367	-23.083	32.693	1.00	63.22	1SG 903
ATOM	903	CA	PHE	117	39.994	-21.971	31.869	1.00	63.22	1SG 904
ATOM	904	CB	PHE	117	38.785	-21.165	32.381	1.00	63.22	1SG 905
ATOM	905	CG	PHE	117	37.636	-22.087	32.611	1.00	63.22	1SG 906
ATOM	906	CD1	PHE	117	37.093	-22.836	31.593	1.00	63.22	1SG 907
ATOM	907	CD2	PHE	117	37.071	-22.168	33.862	1.00	63.22	1SG 908
ATOM	908	CE1	PHE	117	36.028	-23.672	31.833	1.00	63.22	1SG 909
ATOM	909	CE2	PHE	117	36.005	-23.001	34.104	1.00	63.22	1SG 910
ATOM	910	CZ	PHE	117	35.481	-23.761	33.089	1.00	63.22	1SG 911
ATOM	911	C	PHE	117	41.128	-20.994	31.848	1.00	63.22	1SG 912
ATOM	912	O	PHE	117	41.981	-20.988	32.736	1.00	63.22	1SG 913
ATOM	913	N	LEU	118	41.165	-20.135	30.807	1.00	54.77	1SG 914
ATOM	914	CA	LEU	118	42.161	-19.110	30.751	1.00	54.77	1SG 915
ATOM	915	CB	LEU	118	42.137	-18.305	29.440	1.00	54.77	1SG 916
ATOM	916	CG	LEU	118	43.205	-17.199	29.367	1.00	54.77	1SG 917
ATOM	917	CD2	LEU	118	42.964	-16.266	28.170	1.00	54.77	1SG 918
ATOM	918	CD1	LEU	118	44.623	-17.788	29.386	1.00	54.77	1SG 919
ATOM	919	C	LEU	118	41.804	-18.188	31.865	1.00	54.77	1SG 920
ATOM	920	O	LEU	118	40.636	-17.832	32.026	1.00	54.77	1SG 921
ATOM	921	N	CYS	119	42.797	-17.789	32.680	1.00	45.64	1SG 922
ATOM	922	CA	CYS	119	42.468	-16.952	33.792	1.00	45.64	1SG 923

	Atom		Amino Acid		X	Y	Z	Occup.	B	
	No.	Name	SC	No.					Factor	
ATOM	923	CB	CYS	119	42.456	-17.692	35.141	1.00	45.64	1SG 924
ATOM	924	SG	CYS	119	41.139	-18.941	35.241	1.00	45.64	1SG 925
ATOM	925	C	CYS	119	43.469	-15.856	33.900	1.00	45.64	1SG 926
ATOM	926	O	CYS	119	44.536	-15.889	33.286	1.00	45.64	1SG 927
ATOM	927	N	GLY	120	43.114	-14.837	34.704	1.00	39.83	1SG 928
ATOM	928	CA	GLY	120	43.986	-13.728	34.921	1.00	39.83	1SG 929
ATOM	929	C	GLY	120	43.942	-13.439	36.383	1.00	39.83	1SG 930
ATOM	930	O	GLY	120	42.993	-13.777	37.091	1.00	39.83	1SG 931
ATOM	931	N	PRO	121	45.027	-12.883	36.832	1.00	37.35	1SG 932
ATOM	932	CA	PRO	121	45.105	-12.504	38.217	1.00	37.35	1SG 933
ATOM	933	CD	PRO	121	46.285	-13.436	36.359	1.00	37.35	1SG 934
ATOM	934	CB	PRO	121	46.571	-12.613	38.609	1.00	37.35	1SG 935
ATOM	935	CG	PRO	121	47.140	-13.637	37.614	1.00	37.35	1SG 936
ATOM	936	C	PRO	121	44.516	-11.156	38.496	1.00	37.35	1SG 937
ATOM	937	O	PRO	121	44.351	-10.358	37.575	1.00	37.35	1SG 938
ATOM	938	N	ALA	122	44.183	-10.900	39.775	1.00	35.18	1SG 939
ATOM	939	CA	ALA	122	43.625	-9.655	40.214	1.00	35.18	1SG 940
ATOM	940	CB	ALA	122	43.194	-9.684	41.690	1.00	35.18	1SG 941
ATOM	941	C	ALA	122	44.642	-8.568	40.065	1.00	35.18	1SG 942
ATOM	942	O	ALA	122	44.311	-7.452	39.669	1.00	35.18	1SG 943
ATOM	943	N	GLU	123	45.915	-8.866	40.397	1.00	37.20	1SG 944
ATOM	944	CA	GLU	123	46.938	-7.859	40.355	1.00	37.20	1SG 945
ATOM	945	CB	GLU	123	48.206	-8.212	41.148	1.00	37.20	1SG 946
ATOM	946	CG	GLU	123	48.959	-9.419	40.591	1.00	37.20	1SG 947
ATOM	947	CD	GLU	123	50.199	-9.629	41.448	1.00	37.20	1SG 948
ATOM	948	OE1	GLU	123	50.344	-8.898	42.463	1.00	37.20	1SG 949
ATOM	949	OE2	GLU	123	51.018	-10.521	41.098	1.00	37.20	1SG 950
ATOM	950	C	GLU	123	47.349	-7.622	38.941	1.00	37.20	1SG 951
ATOM	951	O	GLU	123	47.181	-8.459	38.055	1.00	37.20	1SG 952
ATOM	952	N	PRO	124	47.857	-6.441	38.732	1.00	40.57	1SG 953

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	953	CA	PRO	124	48.305	-6.070	37.421	1.00	40.57	1SG 954
ATOM	954	CD	PRO	124	47.362	-5.294	39.473	1.00	40.57	1SG 955
ATOM	955	CB	PRO	124	48.254	-4.540	37.361	1.00	40.57	1SG 956
ATOM	956	CG	PRO	124	48.064	-4.096	38.822	1.00	40.57	1SG 957
ATOM	957	C	PRO	124	49.646	-6.635	37.075	1.00	40.57	1SG 958
ATOM	958	O	PRO	124	50.405	-7.003	37.969	1.00	40.57	1SG 959
ATOM	959	N	GLN	125	49.939	-6.694	35.763	1.00	45.04	1SG 960
ATOM	960	CA	GLN	125	51.200	-7.101	35.218	1.00	45.04	1SG 961
ATOM	961	CB	GLN	125	52.344	-6.163	35.634	1.00	45.04	1SG 962
ATOM	962	CG	GLN	125	52.167	-4.733	35.118	1.00	45.04	1SG 963
ATOM	963	CD	GLN	125	53.357	-3.914	35.595	1.00	45.04	1SG 964
ATOM	964	OE1	GLN	125	54.207	-4.410	36.332	1.00	45.04	1SG 965
ATOM	965	NE2	GLN	125	53.419	-2.625	35.167	1.00	45.04	1SG 966
ATOM	966	C	GLN	125	51.600	-8.504	35.563	1.00	45.04	1SG 967
ATOM	967	O	GLN	125	52.794	-8.778	35.681	1.00	45.04	1SG 968
ATOM	968	N	GLN	126	50.655	-9.453	35.711	1.00	51.17	1SG 969
ATOM	969	CA	GLN	126	51.144	-10.792	35.885	1.00	51.17	1SG 970
ATOM	970	CB	GLN	126	50.898	-11.454	37.254	1.00	51.17	1SG 971
ATOM	971	CG	GLN	126	49.458	-11.692	37.682	1.00	51.17	1SG 972
ATOM	972	CD	GLN	126	49.566	-12.461	38.995	1.00	51.17	1SG 973
ATOM	973	OE1	GLN	126	48.972	-12.099	40.009	1.00	51.17	1SG 974
ATOM	974	NE2	GLN	126	50.351	-13.571	38.973	1.00	51.17	1SG 975
ATOM	975	C	GLN	126	50.634	-11.618	34.743	1.00	51.17	1SG 976
ATOM	976	O	GLN	126	49.703	-11.227	34.041	1.00	51.17	1SG 977
ATOM	977	N	PRO	127	51.248	-12.746	34.517	1.00	53.74	1SG 978
ATOM	978	CA	PRO	127	50.915	-13.519	33.350	1.00	53.74	1SG 979
ATOM	979	CD	PRO	127	52.638	-12.925	34.893	1.00	53.74	1SG 980
ATOM	980	CB	PRO	127	52.083	-14.488	33.130	1.00	53.74	1SG 981
ATOM	981	CG	PRO	127	52.971	-14.333	34.379	1.00	53.74	1SG 982
ATOM	982	C	PRO	127	49.574	-14.168	33.367	1.00	53.74	1SG 983

	Atom		Amino Acid		X	Y	Z	Occup.	B Factor	
	No.	Name	SC	No.						
	ATOM	983	O	PRO	127	49.026	-14.406	34.441	1.00	53.74	1SG 984
	ATOM	984	N	VAL	128	49.021	-14.433	32.165	1.00	52.63	1SG 985
	ATOM	985	CA	VAL	128	47.771	-15.125	32.065	1.00	52.63	1SG 986
	ATOM	986	CB	VAL	128	47.027	-14.903	30.779	1.00	52.63	1SG 987
	ATOM	987	CG1	VAL	128	46.517	-13.460	30.743	1.00	52.63	1SG 988
	ATOM	988	CG2	VAL	128	47.962	-15.234	29.609	1.00	52.63	1SG 989
	ATOM	989	C	VAL	128	48.111	-16.571	32.139	1.00	52.63	1SG 990
	ATOM	990	O	VAL	128	49.112	-17.003	31.569	1.00	52.63	1SG 991
	ATOM	991	N	GLN	129	47.295	-17.353	32.875	1.00	48.04	1SG 992
	ATOM	992	CA	GLN	129	47.592	-18.746	33.010	1.00	48.04	1SG 993
	ATOM	993	CB	GLN	129	48.225	-19.127	34.360	1.00	48.04	1SG 994
	ATOM	994	CG	GLN	129	49.603	-18.506	34.600	1.00	48.04	1SG 995
	ATOM	995	CD	GLN	129	49.408	-17.099	35.143	1.00	48.04	1SG 996
	ATOM	996	OE1	GLN	129	50.374	-16.362	35.333	1.00	48.04	1SG 997
	ATOM	997	NE2	GLN	129	48.130	-16.718	35.412	1.00	48.04	1SG 998
	ATOM	998	C	GLN	129	46.317	-19.510	32.904	1.00	48.04	1SG 999
	ATOM	999	O	GLN	129	45.256	-18.948	32.637	1.00	48.04	1SG1000
	ATOM	1000	N	LEU	130	46.415	-20.841	33.084	1.00	42.95	1SG1001
	ATOM	1001	CA	LEU	130	45.265	-21.688	33.032	1.00	42.95	1SG1002
	ATOM	1002	CB	LEU	130	45.526	-22.971	32.223	1.00	42.95	1SG1003
	ATOM	1003	CG	LEU	130	44.261	-23.779	31.898	1.00	42.95	1SG1004
	ATOM	1004	CD2	LEU	130	44.614	-25.141	31.280	1.00	42.95	1SG1005
	ATOM	1005	CD1	LEU	130	43.309	-22.962	31.008	1.00	42.95	1SG1006
	ATOM	1006	C	LEU	130	44.978	-22.048	34.459	1.00	42.95	1SG1007
	ATOM	1007	O	LEU	130	45.856	-22.543	35.166	1.00	42.95	1SG1008
	ATOM	1008	N	THR	131	43.741	-21.780	34.930	1.00	39.85	1SG1009
	ATOM	1009	CA	THR	131	43.402	-22.057	36.298	1.00	39.85	1SG1010
	ATOM	1010	CB	THR	131	42.903	-20.856	37.048	1.00	39.85	1SG1011
	ATOM	1011	OG1	THR	131	43.888	-19.833	37.045	1.00	39.85	1SG1012
	ATOM	1012	CG2	THR	131	42.580	-21.276	38.492	1.00	39.85	1SG1013

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	1013	C	THR	131	42.298	-23.062	36.287	1.00	39.85	1SG1014
ATOM	1014	O	THR	131	41.472	-23.077	35.375	1.00	39.85	1SG1015
ATOM	1015	N	LYS	132	42.263	-23.950	37.301	1.00	42.27	1SG1016
ATOM	1016	CA	LYS	132	41.251	-24.962	37.307	1.00	42.27	1SG1017
ATOM	1017	CB	LYS	132	41.817	-26.364	37.602	1.00	42.27	1SG1018
ATOM	1018	CG	LYS	132	40.802	-27.495	37.414	1.00	42.27	1SG1019
ATOM	1019	CD	LYS	132	41.420	-28.894	37.440	1.00	42.27	1SG1020
ATOM	1020	CE	LYS	132	41.938	-29.308	38.818	1.00	42.27	1SG1021
ATOM	1021	NZ	LYS	132	42.507	-30.673	38.757	1.00	42.27	1SG1022
ATOM	1022	C	LYS	132	40.262	-24.650	38.376	1.00	42.27	1SG1023
ATOM	1023	O	LYS	132	39.497	-25.519	38.791	1.00	42.27	1SG1024
ATOM	1024	N	GLU	133	40.215	-23.392	38.843	1.00	47.60	1SG1025
ATOM	1025	CA	GLU	133	39.251	-23.139	39.864	1.00	47.60	1SG1026
ATOM	1026	CB	GLU	133	39.872	-23.015	41.267	1.00	47.60	1SG1027
ATOM	1027	CG	GLU	133	38.858	-23.133	42.404	1.00	47.60	1SG1028
ATOM	1028	CD	GLU	133	38.080	-21.832	42.478	1.00	47.60	1SG1029
ATOM	1029	OE1	GLU	133	38.641	-20.782	42.066	1.00	47.60	1SG1030
ATOM	1030	OE2	GLU	133	36.913	-21.873	42.951	1.00	47.60	1SG1031
ATOM	1031	C	GLU	133	38.567	-21.856	39.543	1.00	47.60	1SG1032
ATOM	1032	O	GLU	133	39.207	-20.848	39.243	1.00	47.60	1SG1033
ATOM	1033	N	SER	134	37.225	-21.875	39.592	1.00	52.73	1SG1034
ATOM	1034	CA	SER	134	36.485	-20.673	39.383	1.00	52.73	1SG1035
ATOM	1035	CB	SER	134	35.282	-20.840	38.440	1.00	52.73	1SG1036
ATOM	1036	OG	SER	134	34.602	-19.602	38.292	1.00	52.73	1SG1037
ATOM	1037	C	SER	134	35.958	-20.330	40.734	1.00	52.73	1SG1038
ATOM	1038	O	SER	134	35.151	-21.062	41.304	1.00	52.73	1SG1039
ATOM	1039	N	GLU	135	36.440	-19.204	41.283	1.00	55.75	1SG1040
ATOM	1040	CA	GLU	135	36.079	-18.734	42.586	1.00	55.75	1SG1041
ATOM	1041	CB	GLU	135	37.055	-19.230	43.675	1.00	55.75	1SG1042
ATOM	1042	CG	GLU	135	36.567	-19.097	45.120	1.00	55.75	1SG1043

	Atom No.	Atom Name	Amino Acid SC	Amino Acid No.	X	Y	Z	Occup.	B Factor	
ATOM	1043	CD	GLU	135	35.508	-20.164	45.363	1.00	55.75	1SG1044
ATOM	1044	OE1	GLU	135	35.831	-21.369	45.193	1.00	55.75	1SG1045
ATOM	1045	OE2	GLU	135	34.360	-19.787	45.722	1.00	55.75	1SG1046
ATOM	1046	C	GLU	135	36.245	-17.259	42.456	1.00	55.75	1SG1047
ATOM	1047	O	GLU	135	36.592	-16.792	41.377	1.00	55.75	1SG1048
ATOM	1048	N	PRO	136	35.992	-16.488	43.465	1.00	54.05	1SG1049
ATOM	1049	CA	PRO	136	36.189	-15.078	43.306	1.00	54.05	1SG1050
ATOM	1050	CD	PRO	136	34.852	-16.745	44.326	1.00	54.05	1SG1051
ATOM	1051	CB	PRO	136	35.458	-14.409	44.476	1.00	54.05	1SG1052
ATOM	1052	CG	PRO	136	34.896	-15.574	45.316	1.00	54.05	1SG1053
ATOM	1053	C	PRO	136	37.622	-14.661	43.143	1.00	54.05	1SG1054
ATOM	1054	O	PRO	136	37.845	-13.506	42.786	1.00	54.05	1SG1055
ATOM	1055	N	SER	137	38.604	-15.540	43.431	1.00	51.53	1SG1056
ATOM	1056	CA	SER	137	39.993	-15.167	43.361	1.00	51.53	1SG1057
ATOM	1057	CB	SER	137	40.927	-16.312	43.781	1.00	51.53	1SG1058
ATOM	1058	OG	SER	137	40.713	-16.644	45.144	1.00	51.53	1SG1059
ATOM	1059	C	SER	137	40.415	-14.754	41.980	1.00	51.53	1SG1060
ATOM	1060	O	SER	137	40.455	-13.567	41.661	1.00	51.53	1SG1061
ATOM	1061	N	ALA	138	40.745	-15.746	41.123	1.00	51.19	1SG1062
ATOM	1062	CA	ALA	138	41.240	-15.485	39.798	1.00	51.19	1SG1063
ATOM	1063	CB	ALA	138	42.003	-16.671	39.184	1.00	51.19	1SG1064
ATOM	1064	C	ALA	138	40.085	-15.176	38.911	1.00	51.19	1SG1065
ATOM	1065	O	ALA	138	38.958	-15.598	39.168	1.00	51.19	1SG1066
ATOM	1066	N	ARG	139	40.343	-14.422	37.825	1.00	54.85	1SG1067
ATOM	1067	CA	ARG	139	39.256	-14.072	36.968	1.00	54.85	1SG1068
ATOM	1068	CB	ARG	139	39.302	-12.611	36.498	1.00	54.85	1SG1069
ATOM	1069	CG	ARG	139	38.052	-12.197	35.729	1.00	54.85	1SG1070
ATOM	1070	CD	ARG	139	37.915	-10.685	35.568	1.00	54.85	1SG1071
ATOM	1071	NE	ARG	139	37.851	-10.092	36.933	1.00	54.85	1SG1072
ATOM	1072	CZ	ARG	139	36.739	-10.278	37.703	1.00	54.85	1SG1073

	Atom	No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
	ATOM	1073	NH1	ARG	139	35.744	-11.108	37.275	1.00	54.85	1SG1074
	ATOM	1074	NH2	ARG	139	36.627	-9.644	38.907	1.00	54.85	1SG1075
	ATOM	1075	C	ARG	139	39.308	-14.960	35.774	1.00	54.85	1SG1076
	ATOM	1076	O	ARG	139	40.374	-15.205	35.210	1.00	54.85	1SG1077
	ATOM	1077	N	THR	140	38.142	-15.507	35.386	1.00	60.01	1SG1078
	ATOM	1078	CA	THR	140	38.109	-16.363	34.244	1.00	60.01	1SG1079
	ATOM	1079	CB	THR	140	37.824	-17.788	34.614	1.00	60.01	1SG1080
	ATOM	1080	OG1	THR	140	37.863	-18.617	33.463	1.00	60.01	1SG1081
	ATOM	1081	CG2	THR	140	36.450	-17.865	35.293	1.00	60.01	1SG1082
	ATOM	1082	C	THR	140	37.025	-15.874	33.336	1.00	60.01	1SG1083
	ATOM	1083	O	THR	140	36.615	-16.582	32.420	1.00	60.01	1SG1084
	ATOM	1084	N	LYS	141	36.566	-14.624	33.549	1.00	64.19	1SG1085
	ATOM	1085	CA	LYS	141	35.490	-14.050	32.794	1.00	64.19	1SG1086
	ATOM	1086	CB	LYS	141	34.440	-13.403	33.719	1.00	64.19	1SG1087
	ATOM	1087	CG	LYS	141	33.242	-12.749	33.028	1.00	64.19	1SG1088
	ATOM	1088	CD	LYS	141	33.563	-11.464	32.261	1.00	64.19	1SG1089
	ATOM	1089	CE	LYS	141	32.375	-10.893	31.487	1.00	64.19	1SG1090
	ATOM	1090	NZ	LYS	141	31.301	-10.500	32.426	1.00	64.19	1SG1091
	ATOM	1091	C	LYS	141	36.051	-12.986	31.907	1.00	64.19	1SG1092
	ATOM	1092	O	LYS	141	36.909	-12.205	32.319	1.00	64.19	1SG1093
	ATOM	1093	N	PHE	142	35.552	-12.922	30.654	1.00	64.86	1SG1094
	ATOM	1094	CA	PHE	142	36.082	-11.974	29.719	1.00	64.86	1SG1095
	ATOM	1095	CB	PHE	142	36.991	-12.636	28.666	1.00	64.86	1SG1096
	ATOM	1096	CG	PHE	142	38.061	-13.398	29.376	1.00	64.86	1SG1097
	ATOM	1097	CD1	PHE	142	39.036	-12.742	30.088	1.00	64.86	1SG1098
	ATOM	1098	CD2	PHE	142	38.108	-14.772	29.314	1.00	64.86	1SG1099
	ATOM	1099	CE1	PHE	142	40.028	-13.438	30.739	1.00	64.86	1SG1100
	ATOM	1100	CE2	PHE	142	39.099	-15.473	29.963	1.00	64.86	1SG1101
	ATOM	1101	CZ	PHE	142	40.062	-14.809	30.681	1.00	64.86	1SG1102
	ATOM	1102	C	PHE	142	34.952	-11.358	28.950	1.00	64.86	1SG1103

Atom	No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	1103	O	PHE	142	33.832	-11.866	28.939	1.00	64.86	1SG1104
ATOM	1104	N	TYR	143	35.255	-10.226	28.279	1.00	62.77	1SG1105
ATOM	1105	CA	TYR	143	34.355	-9.491	27.432	1.00	62.77	1SG1106
ATOM	1106	CB	TYR	143	34.142	-8.018	27.852	1.00	62.77	1SG1107
ATOM	1107	CG	TYR	143	33.283	-7.851	29.065	1.00	62.77	1SG1108
ATOM	1108	CD1	TYR	143	33.820	-7.825	30.334	1.00	62.77	1SG1109
ATOM	1109	CD2	TYR	143	31.922	-7.698	28.926	1.00	62.77	1SG1110
ATOM	1110	CE1	TYR	143	33.012	-7.655	31.437	1.00	62.77	1SG1111
ATOM	1111	CE2	TYR	143	31.109	-7.527	30.022	1.00	62.77	1SG1112
ATOM	1112	CZ	TYR	143	31.654	-7.505	31.282	1.00	62.77	1SG1113
ATOM	1113	OH	TYR	143	30.819	-7.327	32.407	1.00	62.77	1SG1114
ATOM	1114	C	TYR	143	35.056	-9.394	26.110	1.00	62.77	1SG1115
ATOM	1115	O	TYR	143	36.284	-9.463	26.056	1.00	62.77	1SG1116
ATOM	1116	N	PHE	144	34.291	-9.251	25.005	1.00	60.39	1SG1117
ATOM	1117	CA	PHE	144	34.903	-9.104	23.712	1.00	60.39	1SG1118
ATOM	1118	CB	PHE	144	34.301	-10.001	22.617	1.00	60.39	1SG1119
ATOM	1119	CG	PHE	144	34.118	-11.383	23.144	1.00	60.39	1SG1120
ATOM	1120	CD1	PHE	144	35.166	-12.260	23.324	1.00	60.39	1SG1121
ATOM	1121	CD2	PHE	144	32.849	-11.787	23.484	1.00	60.39	1SG1122
ATOM	1122	CE1	PHE	144	34.937	-13.524	23.820	1.00	60.39	1SG1123
ATOM	1123	CE2	PHE	144	32.614	-13.046	23.977	1.00	60.39	1SG1124
ATOM	1124	CZ	PHE	144	33.661	-13.920	24.144	1.00	60.39	1SG1125
ATOM	1125	C	PHE	144	34.546	-7.710	23.277	1.00	60.39	1SG1126
ATOM	1126	O	PHE	144	33.400	-7.288	23.425	1.00	60.39	1SG1127
ATOM	1127	N	GLU	145	35.504	-6.939	22.727	1.00	57.37	1SG1128
ATOM	1128	CA	GLU	145	35.130	-5.605	22.353	1.00	57.37	1SG1129
ATOM	1129	CB	GLU	145	35.800	-4.489	23.184	1.00	57.37	1SG1130
ATOM	1130	CG	GLU	145	35.176	-4.250	24.565	1.00	57.37	1SG1131
ATOM	1131	CD	GLU	145	35.786	-5.213	25.577	1.00	57.37	1SG1132
ATOM	1132	OE1	GLU	145	36.153	-6.350	25.182	1.00	57.37	1SG1133

	Atom No.	Name	Amino Acid SC	No.	X	Y	Z	Occup.	B Factor	
ATOM	1133	OE2	GLU	145	35.895	-4.818	26.768	1.00	57.37	1SG1134
ATOM	1134	C	GLU	145	35.488	-5.355	20.900	1.00	57.37	1SG1135
ATOM	1135	O	GLU	145	36.283	-4.410	20.648	1.00	57.37	1SG1136
ATOM	1136	OXT	GLU	145	34.961	-6.089	20.023	1.00	57.37	1SG1137

EXAMPLE 15

Determination of IL-1 Hy2 Crystal Structure

Crystallization is used to verify the predicted three-dimensional structure of IL-1 Hy2 using methods known in the are, *e.g.*, as described by Vigers *et al.* (*Nature* 386: 190-194, 1997). Recombinant IL-1 Hy2 and the IL-1 receptor art incubated under conditions which promote binding. The IL-1 Hy2 receptor complex is then purified and crystallized.

Crystals consisting of the IL-1 Hy2 alone. IL-1 Hy2/IL-1 receptor complex or IL-1 Hy2/IL-1 receptor accessory protein or IL-1 Hy2/Il-1R/IL-1 receptor accessory protein are grown by methods known in the art. (*See* Jensen *et al.*, *J. Immunol.* 15: 5277-86, 2000). For example, hanging-drop diffusion against a salt solution such as an ammonium sulphate solution, an ammonium nitrate solution or an ammonium chloride solution may be used to form IL-1 Hy2 crystals. The resulting crystals are diffracted in a beam of x-rays to determine their quality. Heavy-atom derivatives are created and compared to the native crystal. The crystals are cryoprotected and diffracted to determine the diffraction pattern, the unit cell dimensions and symmetry. Molecular replacement is used to determine the final three-dimensional structure.

EXAMPLE 16

Determination of Critical Residues by Site-Directed Mutagenesis

Site-directed mutagenesis is carried out to confirm the location of specific amino acids within the IL-1 Hy2 polypeptide sequence such as those residues predicted to interact with the IL-1 receptor and those predicted to be important to biological function. The mutants are designed based on the predicted three-dimensional structure (described in Example 14) or on the crystal structure (described in Example 15).

Mutants are produced, e.g., via site-directed mutagenesis performed on IL-1 Hy2 cDNA constructs using any method known in the art. For example, uracil-enrichment of single-stranded DNA may be used as described by Kunkel *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 82: 488-492, 1985). Mutagenesis primers are designed based on the (+) coding orientation, and mutagenesis is carried out with a commercially available mutagenesis kit such as the Muta-Gene kit (Biorad) or the Transformer™ Site-Directed Mutagenesis kit (Clontech) according to the manufacturer's instructions.

The mutants generated will have non-conservative substitutions of amino acids predicted to be critical for IL-1 Hy2 function or IL-1 receptor binding, or IL-1 receptor accessory protein binding. In addition, mutants will have amino acid insertions and/or deletions within functional domains, and a single mutant may contain more than one change within the amino acid sequence.

The resulting mutants cDNAs are sequenced, and recombinant IL-1 Hy2 mutants are purified and analyzed in functional assays such as the IL-1 receptor binding assay (described in Example 12), inhibition of IL-6 production assay (described in Example 10) and inhibition of IL-18 activity assay (described in Example 11). Both mutants which knock out function and those that increase function are desirable. In addition, these mutants can be crystallized (as described in Example 15) to determine if a change in the amino acid sequence alters the three-dimensional structure of IL-1 Hy2. Mutants lacking IL-1 Hy2 activity, including receptor binding activity, are useful in screening for compounds which bind to the wild type IL-1 Hy2 polypeptide but do not interact with the IL-1 Hy2 mutants, and thus identifying modulators specific for the active site.

EXAMPLE 17

Expression of IL-1Hy2 in *E.coli*

The coding region of SEQ ID NO: 2 was expressed in *E. coli*. First, the coding region was amplified by PCR using the forward primer QB36 (GTCATATGTGTTCCCTCCCCATGGCAAG; SEQ ID NO: 25) and reverse primer QB38 (GTTTTACTTTGAACAGAGCTGGTAGTGATCAAGCTTC; SEQ ID NO: 26). Primer QB36 corresponds to nucleotides 54 to 76 of SEQ ID NO: 1 and Primer QB38 corresponds to the reverse complement of nucleotides 488 to 512 of SEQ ID NO: 1. The underlined

nucleotides are restriction sites to assist in cloning. PCR was carried out using Pfu polymerase and the primer QB36 and QB38.

The PCR product was ligated into the TOPO cloning vector pCRII (Invitrogen) and transformed into electrocompetent *E. coli* strain Top10 (Invitrogen). The transformed cells were plated on ampicillin-containing plates. Colonies were screened for the correct insert using a PCR reaction employing a gene-specific primer and a vector-specific primer. Positives were then sequenced to ensure correct sequences.

The pCRII was digested with NdeI and HindIII and purified with low melting agarose (LMPA) from FHM Bioproducts. The resulting IL-1 Hy2 fragment was subcloned into the prokaryotic expression vector pRSETB (Invitrogen). The pRSETB was useful for protein expression because it contains an efficient promoter (phage T7) to drive trancription. In addition, this vector provides gene expression control with the lac operator system, which can be induced by the addition of IPTG (isopropyl-β-D-thiogaloctopyranoside).

The pRSETB vector containing IL-1 Hy2 was transformed into *E. coli* strain DH10B (Invitrogen) The transformed cells were plated on carbenicillin-containing plates. Colonies containing the correct insert were verified by restriction digest. Six clones were selected, the DNA was isolated with a mini-prep (QIAgen) and then transformed into the BL21(DE3)plysS cells (Invitrogen). Cloning between the NdeI and HindIII sites resulted in IL-1 Hy2 gene expression without tags.

The transformed BL21DE3pLysS cells containing the plasmid pRSETB with IL-1 Hy2 were cultured in LB broth containing 0.1 mg/ml of ampicillin and 20 µg/ml Chloamphenicol. A 200 ml starter culture was innoculated from with BL21DE3pLysS cells and the culture was allowed to grow overnight at 37°C with constant shaking at 250 RPM. The starter culture was then used to innoculate 4 liters of complex fermenter media containing 0.1 mg/ml of ampicillin. At this point, IPTG was added to a final concentration of 1 mM at 25 OD to induce protein expression. The culture was allowed to grow for 2 more hours, and then the cells were harvested at a final OD_{A600} of 48.5 and a density of 59.6 grams/liter by centrifugation at 10,000 x g for 30 minutes. The cell pellets were stored at -70°C.

EXAMPLE 18

Purification of IL-1 HY2 Polypeptide from *E.coli*

For two 5 liter fermentations, 550 grams of wet cell paste were suspended in 5 liters of lysis buffer (20 mM Tris pH 8.0, 1 mM EDTA). The cells were disrupted with two passes through an Avestin C50 homogenizer at 15, 000 psi. The temperature after disruption was kept at or below 24°C using a cooling coil and ice bath. After disruption, the lysate was centrifuged at 13,000 x g for 20 minutes to remove cell debris. The lysate supernatant was clarified with 1M BisTris (pH 6.0) to reach a final concentration of 20 mM Bis Tris. The supernatant was then titrated to pH 4.7 using 0.5 HCl to precipitate the proteins. The precipitated proteins were removed by centrifugation at 13,000 x g for 30 minutes. The pH of the clarified titrated supernatant was then adjusted to 6.0 using 0.5 M NaOH.

Q-Sepharose Anion Exchange Chromatography at pH 8.0

The pH 6.0 supernatent was loaded onto a 500 ml Q-Sepharose FF column (AP Biotech) equilibrated in 20 mM Bis Tris pH 6.0 (Equilibration Buffer A) at 100 cm/hr linear flow rate. After the sample was applied, the column was washed with 2 volumes of Equilibration Buffer A. Subsequently, the column was washed with two volumes of Elution Buffer A (20 mM Tris pH 8.0) followed by two volumes of 20% Elution Buffer B (20 mM Tris pH 8.0, 500 mM NaCl). The colum was then eluted with a 15 volume linear gradient consisting of 20% to 100% Buffer B. Fractions were collected and evaluated by SDS PAGE. The fractions were collected in two pools based on the SDS PAGE results and a chromatogram.

Phenyl Sepharose High Sub Hydrophobic Interaction Chromatography

The main pool generated by Q-Sepharose chromatography (described above) was titrated to pH7.0 using 2 M NaOH. This pool was then divided into two equal alliquots and ammonium sulfate was added to a final concentration of 1.5 M ammonium sulfate using a 4 M solution. The pool was then loaded onto a 180 ml Phenyl Sepharose High Sub Column (AP Biotech), equilibrated in 1.5 M ammonium sulfate, 50 mM sodium phosphate at pH 7.0 (Equilibration Buffer B), in two runs at 100cm/hr linear flow rate. The column was then washed with five volumes of Equilibration Buffer B. Subsequently, the column was eluted with a 15 volume linear gradient consisting of 1.2 M to 0.45 M amminoium sulfate.

Fractions from the two runs which contained the lowest level of impurities as seen on a SDS-PAGE gel were pooled. The side fractions that were not included in the pool were combined and run as a third aliquot under the same conditions to recover more purified IL-1Hy2.

The combined pools were concentrated approximately 5 fold using a Millipore PrepScale spiral 10 K molecular weight cut off membrane cartridge. Ammonium sulfate was then removed by dilution and diafiltration with 1 L of 50 mM sodium phosphate (pH 7.0).

DEAE Sepharose Anion Exchange Chromatogrpahy

The desalted pool was loaded onto a 75 ml DEAE Sepharose FF column (AP Biotech) in order to remove endotoxins and additional impurities. The column was first equilibrated in 20 mM sodium phosphate pH 7.0 (Equilibration Buffer C). After loading of the sample at a flow rate of 100cm/hr, the column was washed with 5 volumes of Equilibration Buffer C. Subsequently, the column was eluted with 4 volumes of 200 mM NaCl. The protein eluted in one peak and was collected in a single pool.

Q-Sepharose Anion Exchange Chromotography at pH 7.0

The pool from the DEAE Sepharose column (described above) was diluted approximately two-fold with 20 mM sodium phosphate (pH 7.0) to lower conductivity and was loaded onto a 50 ml Q-Sepharose column (AP Biotech) equilibrated with Equilibration Buffer C. The column was eluted with 10 volume linear gradient consisting of 0 to 350 mM NaCl. The eluted protein was collected in 3 peaks and the fractions from the first two peaks were pooled together.

This pool was concentrated to 22.5 mg/ml using an Amicon Stircell with a YM10 membrane. Since the concentration of NaCl in the pool was calculated to be approximately 110 mM and no other formulations were needed. The concentrated pool was sterile filtered and stored at -80°C.

The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous modifications and variations in the practice of the invention are

expected to occur to those skilled in the art upon consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims. All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.